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(FIZIOLOGIYA RASTENII)

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THE DIURNAL RHYTHM OF POTASSIUM TRANSFER FROM THE ROOT SYSTEM TO THE AERIAL ORGANS OF PLANTS

O. M. Trubetskova and N. G. Zhirnova

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Sabinin and his colleagues have developed anew many phases of the physiology of root systems with long-term experiments [1-3]. However, until now there has been but little study of the rhythm of the activities of root systems, knowledge of which is necessary for the development of a theory of the sap transfer of plants and for the resolution of practical questions of plant deficiencies of mineral food elements.

There is a diurnal rhythm in the rate of sap transfer for row plants grown in outdoor conditions which is retained for some time after severing the aerial organs. In all of the study plants, the maximum rate of sap transfer was observed during the daylight hours, and the minimum rate during the dark hours [4-13]. Trubetskova and Shidlovskaya consider that the diurnal rhythm in the rate of sap transfer must be accompanied by a similar rhythm in the transfer of food substances from the root system to the aerial organs [12]. In studying the sap transfer in sunflower, a plant with a clearly observed diurnal periodicity in the rate of sap transfer, the authors confirmed their hypothesis using two mineral food elements: calcium and phosphorous. In the present work, the diurnal path of potassium transfer from the root system was also studied in the sunflower. Taking into consideration the intimate relationship between the work of the root system and the green organs which provide the roots with assimilates, we studied the effect of placing the plants in the dark on the activity of the root system. It had been established earlier that even a nine-day absence from light for leaves of 60-day-old sunflower not only did not retard the sap flow, but did not change its diurnal rhythm [12]. In spite of the reduction in photosynthesis and the consumption of organic substances in the process of respiration, the root system of such large plants as were used in the experiment had probably built up a sizable quantity of respiratory material over a period of time. It is known that in leaves in the dark there is an increase in the decomposition of protein and polysaccharides: products of their hydrolysis can flow toward the root system. Besides this, in the root system itself it is possible that there are processes of reutilization of food substances which guarantee the possibility of growth and of adequately intensive activity of the young parts of the root system. Obviously, the slower the growth and the smaller the size of the plant, the smaller is the amount of light necessary to noticeably reduce the concentration of organic substances in all parts of the plant and thus alter the activity of the roots. A second problem in the present work was to study the change in the activity of the root system of plants of various sizes, depending on the length of time they remain in the dark.

METHOD

The basic experiments were carried out in 1955 with sunflower (type Saratovskii 169). The plants were grown in vegetation containers with 8.1 kg of oven-dry garden soil of the Botanical Garden, Moscow University. Experiment 6 was carried out by student M.G. Moskvitin in 1954, when the plants were also grown on highly nutritive soil in containers with 5.8 kg of oven-dry soil. Six plants were planted in each container.

The plants were irrigated with piped water to 60% of the full water capacity of the soil. Thinning was carried out on the basis of their growth. At the end of the summer in 1954, there were two plants per container, and three plants per container in 1955. Plants of different seeding dates were used in the various experiments. The stems were cut in the morning. On the evening of the day before, the containers with plants were brought into the laboratory where the plants further accumulated sap. In experiments in which the sap was accumulated

TABLE 1

The Effect of Above-Ground Light on the Activity of the Root System of Sunflowers of Different Ages

Expt. no.	Age of plant, in days	Number of leaves	Phase of development	Date of beginning of sap collection	Length of time plants were kept in darkness prior to cutting, days					
					1	2	4	6	8	12
				1955						
1	21	—	Vegetation growth	14.VI	Sap transfer	No trans.	—	—	—	—
2	21	4		4.VIII			—	—	—	—
3	27	4*		20.VI	—		—	—	—	—
4	41	12**	Budding	4.VII	Sap transfer	Sap transfer	Sap trans.	No trans.	—	—
5a	81	20—22	Vegetation growth	23.VIII	—	—		—	Sap trans.	—
5b	91	21—23	Flowering	23.VIII	—	—		—		—
				1954						
6	70	—	Flowering	25.VIII	—	—	—	Sap trans.		Sap trans.

*In the third experiment, for plants kept in the dark for two days, there were 3 leaves.
**In the fourth experiment, for plants kept in the dark for four and six days, there were ten leaves.

for a long period of time, the stump cuts were renewed each day. For studying the effect of exposing the aerial organs to light on the activity of the root system, some of the plants were closed for several days prior to beginning the collection of sap in a light-proof dome in which the temperature was close to that of the air in which the control plants were located.

In each experiment, we determined the rate of sap transfer by two methods: (1) periodic readings (every 15-30 minutes) of the position of the meniscus in a graduated pipette connected to the stump of the plant; and (2) determination of the weight of the sap taken for successive time intervals, and calculation of average rate of sap transfer during the study period. During sap collection, which took from $1\frac{1}{2}$ days to $3\frac{1}{2}$ days, the temperature of the soil in the container with the study plants fluctuated in a range of 1°-2° (with the exception of the third experiment, in which the temperature gradually increased from 12° to 17°).

In the second, third, and fourth experiments with low rates of sap transfer, the sap was collected from four plants together, using two repetitions. In the fifth and sixth experiments, the sap was collected from every plant separately.

Potassium concentration was determined using the method of Kramer and Tisdall [14]. A quantity of 40% formalin was added to the solution to avoid a precipitate of the ammonium ion together with potassium in the sap located in the centrifuge test tube. After heating the solution for a period of 20 minutes at 100°, the NH_4-ion was extracted as a result of the formation of hexamethylenetetramine [15]. After refrigeration of the solution, the regular precipitation of the potassium, cobalt nitrite, and sodium was carried out.

Knowing that the product of the potassium concentration in the sap and the volume of sap produced by one plant per unit of time can be used to characterize the activity of the root system by its supply of these or other nutritive substances to the aerial organs, we calculated this amount for potassium. It is expressed in micrograms of potassium transported in the sap of one root system in one hour.

RESULTS OF THE EXPERIMENTS

In Table 1, we give characteristics of plants and the results of experiments on the effect on the activity of the root systems of the various times that plants of different sizes are kept in the dark.

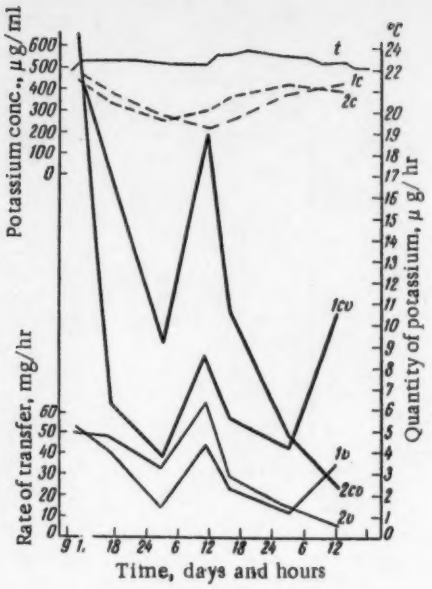

Fig. 1. The effect of keeping plants in darkness on the activity of the root system of 23-day-old sunflower (Expt. 2).
1v, 2v) Rate of sap transfer; 1c, 2c) potassium concentration in the sap; 1 cv, 2cv) quantity of potassium transferred bythe sap of one plant; 1v, 1c, 1cv) control plants; 2v, 2c, 2cv) plants kept in the dark one day; all curves are the average of two parallels; t) soil temperature.

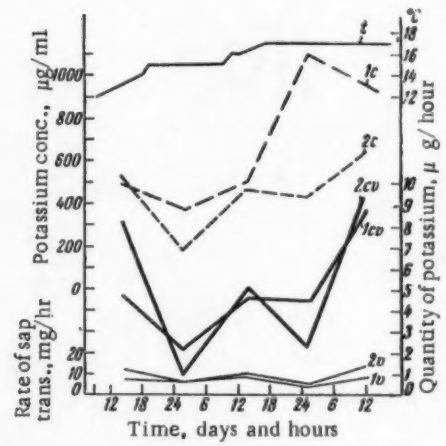

Fig. 2. Diurnal path of root system activity for 27-day-old sunflower (Expt. 3).
1v, 2v) Rate of sap transfer; 1c, 2c) potassium concentration in the sap; 1cv, 2cv) quantity of potassium transferred by the sap of one plant; 1, 2) parallel tests; t) soil temperature.

From the data in Table 1 we can see that the younger and smaller the plant, the shorter the period of time in the dark necessary for the change in the work of the root system. Thus, 21-27-day-old plants lost the ability for sap transfer after just two days absence from light for the aerial organs, and the 41-day-old plants after only six days. For 81-91-day-old plants, sap transfer was not stopped until after eight days, and for large 70-day-old plants in 1954 not even after 12 days in the dark.

The results of the second to the fifth experiments for the study of the diurnal path of the rate of sap transfer, the transfer of potassium by the root systems, and its concentration in the sap of control and study plants is represented on graphs (Figs. 1-6). All graphs show the marked divergence in the data of the two biological repetitions. One curve, drawn for the average quantities, is derived from the closely matching parallels. The rate of sap transfer (mg/hour) is given only for the data of the fractional collection of sap. Not cited in the work, but used in consideration of the results, are the curves of the rate of flow obtained by means of hourly readings of the position of the meniscus in the capillary, which confirmed and checked the data cited on the graphs.

Sunflowers of an age of 23-27 days (Figs. 1 and 2) have a clearly defined diurnal rhythm in the rate of sap transfer, which accompanies a similar rhythm in the transfer of potassium by the root system. The average rate of sap transfer and the quantity of potassium transferred by the root system during the day are 1½-3 times as large as during the night. The real maximum figure for the rate of sap transfer obtained in the first experiment was 5-6 times as large as the average rate of flow during the night.

For 23-day-old plants of the second experiment, the rate of sap transfer and the quantity of potassium in the sap was greater than for the 27-day-old plants in the third experiment, and this is explained by the better growth conditions of these plants and the higher temperature during the time of sap collection.

In the second experiment, 23-day-old plants lost the ability for sap transfer after being kept in darkness for two days, but the absence of light for only one day not only did not decrease, but even increased the rate of sap transfer and the amount of potassium transferred by the root system to the aerial organs both during the day and at night. However, for experimental plants, in contrast to the controls, the rate of flow and amount of potassium transported with the sap was not increased on the third day after cutting.

The relation of the rate of sap transfer and the transfer of potassium on the first and second days to the rate of these processes during the night of the first day for plants kept in darkness are the same as those for the control plants.

In the third experiment during the first day and a half, the concentration of potassium in the sap was greater

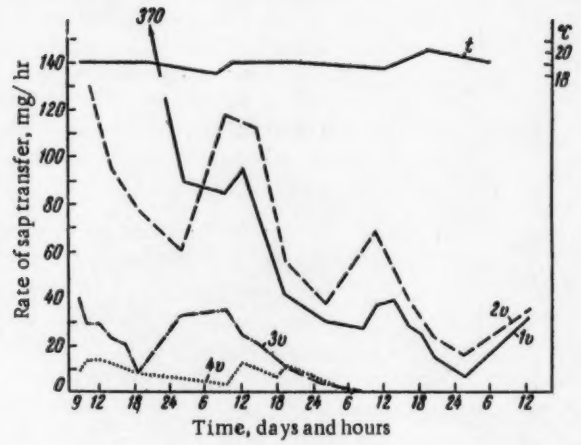

Fig. 3. The effect of holding plants in the dark on the rate of sap transfer for 41-day-old sunflower (Expt. 4).
1v) Control plants; 2v) plants kept in darkness for two days; 3v, 4v) plants kept in darkness for four days; 1 and 2) average of two parallels; 3 and 4) parallel tests; t) soil temperature.

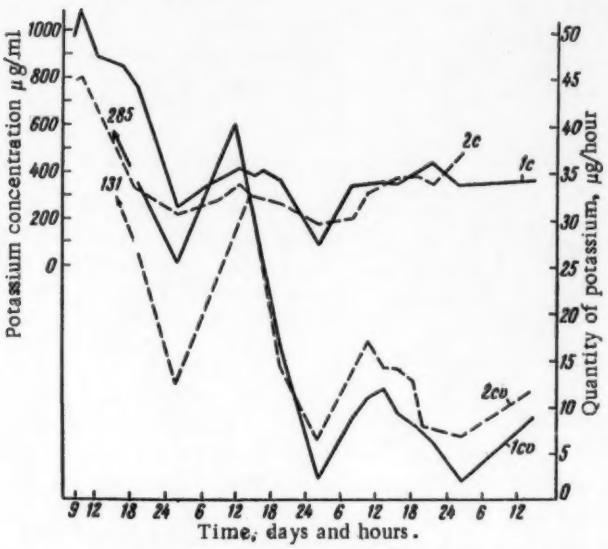

Fig. 4. The effect of keeping plants in the dark on the concentration and quantity of potassium in the sap of 41-day-old sunflower (Exp. 4).
1c, 2c)The concentration of potassium in the sap; 1cv, 2cv) the quantity of potassium carried with the sap by one plant; 1c, 1cv) control plants; 2c, 2cv) plants kept in the dark two days; the curves are the average of two measurements.

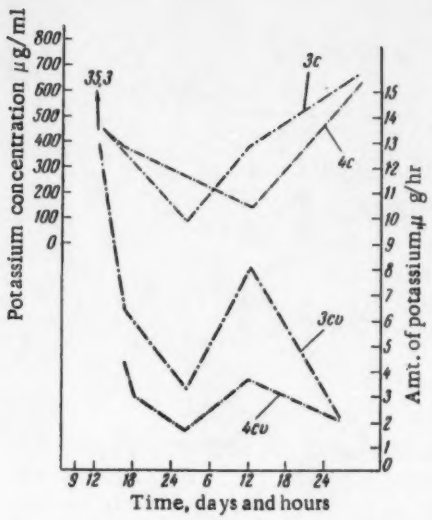

Fig. 5. The concentration and quantity of potassium in the sap of 41-day-old sunflowers kept in the dark four days (Exp. 4). Rate of sap transfer for these plants is seen in Fig. 3.
3c, 4c) Potassium concentration; 3cv, 4cv) amount of potassium transferred in the sap of one plant; 3 and 4) parallel tests.

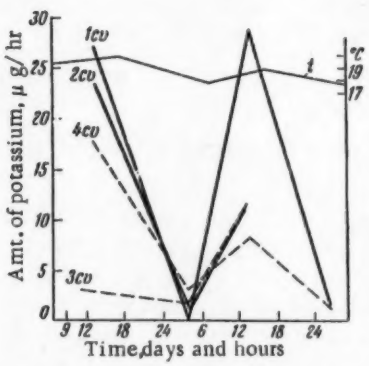

Fig. 6. The effect of keeping plants in darkness on the amount of potassium transferred in the sap of 81-day-old sunflowers (Exp. 5a).
1cv, 2cv) Amount of potassium transferred in the sap of control plants; 3cv, 4cv) amount of potassium transferred in the sap of plants kept in darkness eight days; t) soil temperature.

during the day than at night, that is, it had a diurnal periodicity; but, in the second night, a reduction in the rate of sap transfer accompanied a noticeable increase in the potassium concentration.

In the fourth experiment, prior to the cutting of aerial organs of 41-day-old plants, a portion of them were placed in the dark for two, four, and six days. With a more exact study of the diurnal path of potassium transfer by the root system as the goal, sap was collected every 2-3 hours at night instead of two times per day. The rate of sap transfer of plants of all variants is given in Fig. 3; the concentration and quantity of potassium in the sap of control plants and of those kept in the dark for two days is given in Fig. 4, and for plants kept in the dark for four days in Fig. 5.

In spite of several fluctuations in the potassium concentration in the sap during the day, curves of the quantity of potassium transferred by the root system are, as a rule, smooth and without peaks. Similar results for previous experiments clearly express the diurnal rhythm in the rate of sap transfer and amount of potassium transferred with the sap. The relation of the rate of potassium transfer in the second and third days to the rate of its transfer in the night is greater than in the previous experiments. It is equal to 6-20 times that of control plants and those kept in the dark for two days. The larger amount is not only a result of the more divided collection of sap, but also a result of the greater age of the plants.

This amount is not calculated for the first day because, after cutting the plant, a very sudden decrease in the rate of sap transfer and potassium transfer is observed.

Keeping 41-day-old plants in darkness for two days did not have a noticeable effect on the activity of the root system, but the absence of light for a period of four days prior to cutting the stem, sharply reduced the rate of sap transfer in the first and second days and brought it to a halt after 48 hours. The quantity of potassium transferred by the root system with the sap of plants kept in darkness for four days is 5-10 times as low as that of control plants. But in spite of this, the diurnal rhythm in the rate of sap transfer and the amount of potassium near the minimum for plants of this variant is less than that for the control plants.

As shown above, there was no sap transfer at the start for plants kept in the dark six days. But 36-40 hours after cutting, sap flow started for some of the plants, although it is true that its rate was 10-20 times less than the rate of sap transfer in control plants.

In the fifth experiment there were two groups of plants, 81 and 91 days old. The plants of the first group (experiment 5a) were in the vegetative state because of the removal of their buds; plants of the second group (experiment 5b) were flowering up to the time of sap collection. Part of the plants from each group were kept in the dark for four and eight days. In spite of large individual fluctuations because of the fact that in the relatively

TABLE 2

The Concentration and Amount of Potassium in the Sap of 81-Day-Old Sunflower Plants

Variant	Potassium concentration μ g/ml			Amount of potassium per plant μg/ hr		
	First day	First night	Second day	First day	First night	Second day
Control	404	141	160	24.5	5.0	18.9
Without light:						
Four days	258	104	163	9.5	1.0	17.2
Eight days	217	83	91.6	5.8	1.4	8.4

small containers there were three plants, all, with no difference between flowering and nonflowering plants, had the same rhythm in the activity of the root system that was noted earlier. With from 4 to 13 multiple repetitions in the experiment, in Fig. 6 we show only the data for several plants (two control and two experimental plants) which are typical, and, for the remainder, averages of the sap analysis are given in Table 3.

The exceptionally large difference between the activity of the root system during the day and during the night must be noted. The rate of sap transfer for control plants was sharply reduced during the night hours, often approaching zero (for five out of thirteen plants). It is possible that the sap transfer of these plants was negative during the night; that is, there was a sucking-in of water from the stump of the plants, as we noticed in the 1954 experiments for 70-day-old plants. The cessation of the positive transfer during the night changed on the second day to the secretion of sap with a rate somewhat greater than on the first day after cutting. Keeping plants in darkness for four and eight days did not eliminate the normal rhythm of the work of the root, but decreased both the rate of sap transfer and the amount of potassium transferred by the root system. As is seen in Table 2, the amount of potassium transferred with the sap of the plants kept in darkness for four days was approximately by one third as large as for the control plants, and for plants kept in darkness eight days, the difference was still greater. The potassium concentration in the sap of the study plants was smaller than that in the sap of the controls.

In the 1954 experiments with very large 70-day-old plants, the absence of light even for as long as 12 days did not significantly change the activity of the root system.

It should be noted in passing that the potassium concentration in the sap of flowerless plants was twice as high as in plants with flowers.

The relation of the amount of potassium transferred with the sap during the day to the amount transferred at night increased with the age of the plant. Thus, for 23-27-day-old plants the relationship was 1.5-3, for 41-day-old plants 6-20, at the age of 81-91 days it reached an average of 15-30, and in the absence of positive sap transfer at night it became equal to infinity. Along with this, for old plants the amount of potassium transferred by the roots to the aerial organs per day was not greater than for 41-day-old plants.

The presence of a diurnal rhythm in the transfer of potassium, calcium, and phosphorous by the root system to the aerial organs, which we demonstrated for sunflower, together with the data of Scoog and his co-workers [7] on the electric conduction of sap leads to the conclusion that those plants which under constant external conditions have a diurnal rhythm in the rate of sap transfer have a similar rhythm in supplying the aerial organs with all the elements of mineral food.

Hanson and Biddulph [17] determined the absorption of radioactive phosphorous and its distribution in kidney bean plants at different times of the day. Under constant conditions of temperature and humidity in the dark, the amount of P^{32} absorbed from the surrounding environment in the day and night was the same, but its distribution between aerial organs and roots was different. There was twice as much phosphorous in the aerial organs from 6A.M to 4P.M. as there was during the remainder of the day. Consequently, a diurnal periodicity was discovered in the entire plant in the supplying of the aerial organs with phosphorous, similar to that which we demonstrated for the isolated root system in connection with three elements of mineral food: potassium, calcium, and phosphorous.

Under natural conditions of plant growth, changes in soil temperature throughout the day increase the difference in the amount of nutritive substances transferred by the root system to the aerial organs during the day and at night. Therefore, in using the method described by Sabinin [18] of diagnosing mineral nutrients by analysis of the sap, it is necessary to know the diurnal path in the activity of the root systems of plants at various phases of development. The time and length of sap collection cannot be correctly chosen without a study of this.

In all of our experiments with sunflower of various ages, the paths of the curves of the amount of potassium, calcium, and phosphorous transferred with the sap at various times of the day are analagous to the paths of the curves for the rate of sap transfer. But the relationship of the maximum rates of sap transfer to the minimum, as a rule, is less than the corresponding relationship of the rates of transfer of mineral substances with the sap. The quantity of elements of mineral food transferred with the sap during a given time by the root system is determined by the rate of their secretion by the living cells of the roots into the vessels of the xylem. Therefore, for the period of a day, the rate of sap transfer, that is, the rate of secretion of water into the vessels of the xylem of the root, changes less than the rate of secretion of mineral elements. The rhythm of the latter process is maintained after the removal of the aerial organs for a longer period of time than the rhythm of the rate of sap transfer. Thus, in the second experiment, the rate of sap transfer did not increase on the third day after cutting and the rhythm of transfer of potassium was maintained.

In this way the study of the diurnal periodicity in the activity of the root system leads to the conclusion that under constant external conditions there is not a proportionality between the rate of movement in the root system of water and mineral substances. This regularity was established earlier [19, 20] through changes of several factors in the external environment: temperature and osmotic pressure of the external solution.

However, in spite of the different rates of the two processes, the transfer of water and the transfer of elements of mineral nutrients by the root system to the aerial organs, a concurrence in the time of occurrence of the maximum and minimum rates of both processes leads to the conclusion that there is a single reason for their diurnal rhythm.

The maintainence of a rhythm in the work of the root system over a very long period of time after cutting the aerial organs (up to 22 days), and also the elimination of the effect on the plant of the changes from day and night, and the diurnal periodicity of the root pressure and intensity of respiration of the isolated growing of the root discovered by White [8], force a search for the reasons for the diurnal rhythm in the features of the physiology of the root system itself. The rhythm of the fission of cells of the meristem of the root and their transition to the phase of enlargement, for which there are characteristically changes in the properties of plasma, and the increased intensity of respiration [21] and activity of the series of ferments [22] can show the reason for the diurnal rhythm in the activity of the root system.

SUMMARY

1. Under constant external conditions for sunflowers of different ages there is a diurnal rhythm in the transfer of potassium by the root system to the aerial organs with a maximum during the daylight hours and a minimum during the night hours.

2. The smaller the plant, the shorter the period of keeping the plants in darkness necessary to retard the ability of the root system for sap transfer.

3. After keeping the plants in darkness up to the time that the sap transfer of the plants is maintained, the diurnal rhythm of potassium transfer with the sap is maintained.

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THE TRANSLOCATION OF ASSIMILATES IN WHEAT SEEDLINGS IN CONNECTION WITH ROOT NUTRITION CONDITIONS

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The question of the effect of root nutrition conditions of plants on the translocation of assimilates has remained quite inadequately studied up to the present. In this area of work, almost everyone has used,without change,the original methods of quantitative study of the translocation of substances, but the conclusions are given on the basis of different indirect indicators: the quantity of harvest, the accumulation of sugar, starch etc. in the individual plant organs [1-4].

However, opinions concerning the flow of assimilates based only on these indicators can frequently be in error; for example, the size of the yield, the accumulation of carbohydrates and proteins in the leaves, petioles, stems, roots and tubers are determined not only by the activity of translocation, but also by the intensity of photosynthesis, respiration of the individual organs, the direction of biochemical transformations, etc.

Greater possibilities for the study of the effect of root nutrition conditions on the translocation of organic substances are available in connection with the use of tracer atoms which, though studied and practical in this way, have been little publicized [5-8].

In our experiment in 1956-1957, the problem was to explain the effect of root nutrition conditions on the flow of assimilates from the leaves to the roots of wheat seedlings.

Sugars, moving from the leaves into the root, as established by Kursanov [9-11], and subjected to oxidizing in the roots by the Krebs cycle with the formation of various organic acids, play one of the central roles in the metabolism of the root substances and are connected closely with the assimilation of nitrogen, carbon dioxide, and, it appears, in general with the absorbing activity of the root system [5,8, 11].

Therefore, the resolution of the problem given in our experiments, that is, the explanation of the relation of the flow of assimilates to the absorbing activity of the roots, is quite interesting both from the theoretical relationships and from a purely practical point of view, for the more successful application of fertilizers.

METHODS

The basic plant in the study is wheat, type Lyutestsens 62, and sunflower, type Saratovskii-Early, was used in only one experiment for a comparison.

Two to three-day-old seedlings of these cultures were placed in a Knop solution of the following variants: 1) complete mixture; 2) mixture excluding potassium; 3) mixture excluding nitrogen; and 4) Knop mixture with KCl replaced by $KHCO_3$ in equivalence to the quantity of K_2O. In the latter variant, the effect of root assimilation of carbon dioxide on the translocation of assimilates was studied [5, 9-13]. In all solutions the pH was in the range 6.6-6.8. The seven to fourteen-day-old plants of all variants were placed in a container with $C^{14}O_2$. For this the roots of the seedlings were located in a jar with the food mixture of the appropriate variant and closed with rubber stoppers with cuts for the stems. In order to completely remove the possibility of the dispersion of $C^{14}O_2$ from the containers in the food solution with roots, the cuts in the stopper for the stems were tightly closed with cement. $C^{14}O_2$ from a mixture of 10 μC to 1 liter of volume of the container with the normal concentration of CO_2, 0.3%, was introduced into the container. The exposure of plants in the container in most experiments lasted 30 minutes and in a few cases only, for 60 minutes.

After this, the plants were removed from the container with $C^{14}O_2$, half of them in each variant was fixed immediately in a dessicator at 105°, and the other half was placed in the light and fixed in some experiments after 3 hours and in others, 24 hours after the first fixation.

Prior to the fixation, the leaves were cut from the roots. The dried material was pulverized and placed on a disk (10 mg per cm^2 of disk area) for measuring the radioactivity on a face meter. The coefficient of self-absorption was introduced into the calculations.

Kursanov [14] determined that the sugars can be translocated in the plant in the form of phosphorous esters. Kazaryan [15] showed in his experiments that the translocation of sugars in the plant in a number of cases is connected with the translocation of phosphorous and the latter can serve in this manner as an indicator of the flow of sugars. Several other authors have also reached similar conclusions [16]. At the same time there are also indications of a different nature in the literature concerning the simultaneous meeting of the transfer of phosphorous and assimilates in the phloem [17]. Insofar as the question of the important correlation between the translocation of phosphorous from the leaves and the ordinary current of organic substances is of undoubted interest, in the second series of our experiment the intensity of the flow of P^{32} from the leaves into the roots was determined for those variants of root nutrition (with the exception of the variant with $KHCO_3$, which was not included here). In the leaves, P^{32} was introduced by means of vacuum-infiltration of a 1% solution of $KH_2P^{32}O_4$ (with the activity of 2.5 μC/liter of solution) for a period of 5 minutes. After this the solution of $KH_2P^{32}O_4$ was removed from the surface by quickly eroding with a jet of water and the seedlings were again placed in Knop solutions of the appropriate variants. For removal of the excess water from the intercellular spaces the experimental plants were placed under a ventilator for 15-30 minutes. After the specified lengths of time (3 hours and 24 hours) the leaves and roots were removed and fixed in a dessicator at 105°. After drying, the material was pulverized and placed on a disk (10 mg/cm^2 to determine the radioactivity.

G. Zvonkov, L. Katyushin, L. Kuznetsov and Z. Kirsanov took a direct part in carrying out the experiments.

TABLE 1

Radioactivity of Roots of Wheat and Sunflower During 30-60 minutes After the Beginning of the Exposure in a Container with $C^{14}O_2$ (in imp/min·g dry wt)

Experiment No.	Age of plants days	Complete Knop solution	Without potassium bicarbonate	Without potassium	Without nitrogen
	Wheat				
1	7	1 468	1 653	625	723
2	14	22 700	22 900	22 700	18 350
3	14	1 430	2 530	560	1 030
4	10	300	1 030	0	500
5	14	2 582	—	1 360	1 355
6	10	1 820	3 470	720	960
	Sunflower				
7	7	334	834	328	221

EXPERIMENTAL DATA

The intensity of the flow of assimilates from the leaves to the roots was determined by the radioactivity of roots after the first fixation (that is, during the 30-60 minutes after the beginning of the exposure with $C^{14}O_2$ in the container). In this time, a portion of the assimilated C^{14} (5-20% in most experiments) succeeded in being transferred to the roots and made it possible to make a definite conclusion as to the intensity of translocation of the assimilates. In this connection our results agreed with those of the investigations of Pristupa and Kursanov [8].

As seen from Table 1, in all experiments the radioactivity of the roots during the 30-60 minutes in the variant with $KHCO_3$ was higher than for seedlings in the control, that is, in the ordinary Knop solution. In variants with potassium and nitrogen removed from the food solution, on the other hand, the radioactivity of the roots in nearly all cases was lower than for the control plants. However, it would not be accurate to use the radioactivity of the roots per unit of dry weight to give results for the intensity of translocation of assimilates to the roots from the leaves. It is quite obvious that the accumulation of C^{14} in the roots would be determined also by the intensity of photosynthesis in the given variant, that is, by the normal quantity of $C^{14}O_2$ absorbed by plants and the differences in the relation of the mass of leaves and roots in the variants.

In order to remove the effect of these factors, we determined the normal radioactivity of the roots (product of the activity of 1 g and the dry weight) as a percent of the normal radioactivity of all plants, which was measured by summing the normal radioactivity of the roots and leaves (Table 2).

TABLE 2

Normal Activity of the Roots as Percent of Normal Activity of the Plant

Experiment No.	Complete Knop solution	With potassium bicarbonate	Without potassium	Without nitrogen
1	19.60	17.20	9.80	17.30
2	3.81	4.51	2.32	2.77
3	7.10	10.00	3.80	6.30
4	2.44	8.04	0	6.20
5	1.91	—	1.74	1.87
6	9.14	16.70	5.80	7.62
7	19.70	30.50	8.60	14.50

TABLE 3

Activity of Roots after the Second Fixation: i.e., at 3 and 24 hr after Exposure in a Container with $C^{14}O_2$ (imp/ min/ g dry wt)

Experiment No.	Complete Knop solution	Without potassium bicarbonate	Without potassium	Without nitrogen
Duration of experiment, 24 hours				
1	1 728	2 136	1 436	1 488
2	18 000	20 500	40 700	20 300
Duration of experiment, 3 hours				
3	1800	7760	5400	2820
4	1700	6460	2250	4250
5	9215	—	4150	1870
6	4270	8330	4210	4460
7	2100	1788	2950	3158

TABLE 4

The Dry Weight of Roots as a Percent of the Dry Weight of the Entire Plant

Experiment No.	Complete Knop solution	With potassium bicarbonate	Without potassium	Without nitrogen
1	12.5	21.3	15.1	15.1
2	19.1	20.5	16.8	17.9
3	21.7	24.1	16.0	23.7
4	15.3	18.9	18.9	18.0
5	12.1	12.3	14.3	16.0
6	15.8	20.8	15.0	18.3
7	12.7	12.9	11.5	13.6

The differences between the variants measured in this way also maintained their previous character: in the variant with potassium bicarbonate the radioactivity of the roots as a percent of the normal radioactivity of the plant was at a maximum, and in the variants in which the potassium and nitrogen were removed, this percentage relationship was lower than in the control, that is, the variant with a complete food solution. The flow of assimilates from the leaves to the roots for the food solution without potassium was retarded to a greater degree than in the seedlings lacking in nitrogen.

In a previously published work [13], we described the proposition that the more intensive assimilation of carbon dioxide from the air by the plants receiving potassium bicarbonate as an additional source of carbon dioxide for the roots, can be a result of increased synthesis of amino acid in the roots [8-11]. This can explain the vigorous consumption of carbohydrates in the roots and the formation of organic acids from them as a result of which, as we proposed, the intake of new assimilates into the roots from the leaves is activated. The results of the experiments with the utilization of radioactive carbon cited in the present work supports this proposition.

From work available in the literature on the flow of assimilates, it follows that nitrogen, which aids in the growth of leaf blades and increases the life span of leaves, can decrease the intensity of the flow of assimilates from the leaves [18]. The results we obtained on the direct determination of the intensity of the flow show that, at least for young seedlings, it is otherwise – instead, nitrogen speeds up the translocation of assimilates from the leaves to the roots. In the experiments of Pristupa and Kursanov [8] the short-duration feeding of a solution of NH_4NO_3 to 22-day-old pumpkin plants that had been kept on distilled water for two days prior to this also activated the movement of assimilates to the roots. The authors admitted that one of the reasons for the weak flow of assimilates on nitrogen food is the retarding of the synthesis of amino acids in the roots.

In regard to potassium, the opinion had already been put forth long ago in the literature that it aids in some manner in the translocation of carbohydrates in the plant, in particular in the flow of assimilates from the leaves [1,2,19]. However, this opinion up to this time was based in the main part on such circumstantial evidence as the concentration of sugars and other carbohydrates in the leaves, petioles, stems, tubers, and roots. In our experiments, this position was confirmed by the direct determinations of the intensity of the flow of assimilates.

On increasing the time of flow to 3-24 hours, the characteristic picture of the difference in radioactivity of roots between the variants with the complete solution and without potassium and without nitrogen was lost (Table 3). It can be assumed that one of the reasons for this was the consumption of $C^{14}O_2$ by the roots in the process of respiration.

TABLE 5

The Intensity of Translocation of P^{32} From the Leaves to the Roots

Plant being investigated	Time of introduction of $KH_2P^{32}O_4$ in the leaves up to fixation, hours	Activity of root, imp per min· g dry weight		
		Comp. soln.	Without N	Without K
Four-week-old wheat	24	842	552	761
Three-week-old wheat	3	735	380	270
Same	24	955	930	290
Two-week-old sunflower	3	6810	2130	2216

According to the data of Zalenskii [20], for example, 45-day-old plants of barley consumed in respiration during the first day more than half of the C^{14} assimilated in 40 minutes in the container with $C^{14}O_2$. For other intensities of respiration of the roots, their radioactivity would decrease to various degrees as a result of formation of $C^{14}O_2$ for each variant. This can distort, to a considerable degree, the data obtained on the flow of C^{14} from the leaves to the roots. From this, it follows that on studying the translocation of organic substances using C^{14} the duration of the experiment is necessarily shortened.

The conclusion in the works of several authors on the translocation of assimilates are based on a comparison of the dry weight of leaves, stems, roots, and other organs of the plants [1,2]. The results of our determinations suggest the lack of foundation and inaccuracy of this type of conclusion. Thus, the relationship of the dry weight of roots to the dry weight of the whole plant in the variant without nitrogen was almost always larger than for plants on the complete Knop solution (Table 4). The flow of the assimilates from the leaves to the roots in the variant without nitrogen was, as noted above, less intensive than in the control plants. It also appears that in this case the consumption of assimilates in respiration plays a large role. In the literature, it is indicated that the losses in dry weight for plants through the process of respiration often make up to 20-50% of the normal increase in dry weight at the expense of photosynthesis and, therefore, can have a serious effect on the weight of the plant and its individual organs [21].

The experiments using P^{32} gave results completely analagous to the experiment with $C^{14}O_2$ (Table 5). The translocation of phosphorous from the leaves to the roots in variants in which nitrogen or potassium were excluded was less intensive than for plants on the complete food solution. These data can be considered as positive confirmation of the conclusion we made in the experiments with $C^{14}O_2$. On the other hand, they suggest the existence in several cases of the link between the translocation of phosphorous from the leaves and the normal flow of assimilates.

SUMMARY

1. For wheat seedlings grown on a Knop food solution with nitrogen removed, the translocation of assimilates from the leaves to the roots is less intensive than for seedlings on a complete food solution. The flow is retarded to an even greater degree when potassium is excluded from the food mixture.

2. Replacing potassium chloride in the Knop solution with potassium bicarbonate as an additional source of amino acid for the roots leads to an increased flow of assimilates to the roots of the study seedlings.

3. In experiments using C^{14} for the study of the translocation of organic substances in plants, the time between the entry into the plant of C^{14} and fixation of its individual organs must be reduced as much as possible in order to study radioactivity. If not, the data can be significantly altered as a result of, for example, the consumption of $C^{14}O_2$ in respiration.

4. There is no direct relationship between the intensity of the flow of assimilates from the leaves to the roots and the relationship of the dry weight of these organs.

5. The difference between the variants in the rate of translocation of P^{32} from the leaves to the roots had in our experiments, generally the same character as the differences in the translocation of C^{14}.

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A STUDY OF WATER METABOLISM OF PLANTS USING WATER CONTAINING HEAVY OXYGEN, H_2O^{18}

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Heavy-oxygen-containing water has not been used up to the present time as an indicator in the research on water metabolism of plants. For this purpose, although rather seldom, either deuterium oxide DNO or tritium oxide TNO has been used in previous work [1-4]. Isotopes of hydrogen (D,T) used in the investigations referred to are sharply differentiated by their mass from the normal, light hydrogen (2 and 3 times, respectively for deuterium and tritium). Therefore, it is entirely natural that there were doubts expressed repeatedly in the identity of the behavior of these isotopes in biological systems. In fact, direct experimental data confirming these doubts were obtained in a succession of cases [5,6].

The difference in the mass of heavy oxygen O^{18} from its normal isotope O^{16} was distinctly less pronounced; therefore, when used as an indicator of H_2O^{18}, it seemed that it could avoid,to a significant extent,the undesirable results in biological investigations of the isotope effect.

On the other hand, results of experiments with heavy-oxygen-containing water could be profitable for a comparison and appraisal of data obtained with the aid of deuteriumoxide and tritium oxide.

The present work was set up to explain the rates and dimensions of the assimilation and distribution of H_2O^{18} in the cells of various organs of kidney bean plants. At the same time it was decided that with these experiments some light could be thrown on the question of the irregular distribution of the heavy isotopes of hydrogen between the plants and the food solutions, as disclosed in the experiments using tritium oxide and deuterium oxide [1,2]. This question appeared earlier in the scientific literature; however, up to this time, a rational answer has not yet been found.

EXPERIMENTAL PART

Material: Kidney bean, Phaseolus vulgaris, and bean, Phaseolus aureus, were the plants used in the research.

Seeds of Phaseolus vulgaris were germinated first in quartz sand and then, after the appearance of the two primary leaves, the roots were carefully removed from the sand and the plants were transplanted into a $^1/_5$ Knop food solution. Further sprouting was carried out in the greenhouse at a temperature of 20-25° C up to the time of complete opening of the two primary leaves (Table 1) or to the later stages (Table 2) when the first trilobed leaf unfolded and the second trilobed leaf appeared. At this time the plants had well-developed root systems.

After this the plants were taken from the food solution, the roots were carefully dried with a sheet of filter paper and then the roots of the plants were again immersed in a Knop food solution; however, this time the water was prepared with enriched H_2O^{18}.

Because the experiments were in a chamber, warm air from a fan was blown on the leaves from time to time to speed the transpiration.

After the appropriate exposure, the plants were removed from the food mixture, the roots were again carefully dried with a piece of filter paper and the plants were then separated into roots, stems, and leaves. These

parts were used for the isotope analysis of the water held in them. In one series of experiments (Table 2) the stem was divided into upper and lower halves, first trilobed leaves and second trilobed leaves. Five to six plants were used for every experiment.

Part of the Phaseolus aureus was grown from the seeds to seven days old on a Knop food solution, prepared with water enriched with H_2O^{18}, in a dark constant temperature chamber at 30°. The other part was also grown from seeds on a food solution containing H_2O^{18}, but in the light under daylight lamps (2500 meter-candles) for a period of nine days. An isotope analysis of the water taken from the plants was then made.

TABLE 1

The Assimilation of H_2O^{18} in the Various Organs of Seven and Eight-Day-Old Plants of Phaseolus vulgaris*

Expt. No.	Exposure	Atom % of excess O^{18} in the water		
		root	stem	leaf
1	30 min	0.52	0.14	0.02
2	1 hr, 40 min	0.63	0.60	0.10
3	6 hr	0.67	0.95	0.53
4	12 hr	0.53	0.80	0.62
5	24 hr	0.54	0.77	0.65

* Concentration of H_2O^{18} in the food solution was equal to 0.99 atom % of the excess O^{18}.

Isotope analysis of the water: In order to determine the concentration of O^{18} in the water held in the various organs of the plant, the water was distilled from the appropriate organs by freezing and drying these organs in a vacuum [7]. Oxygen of the water distilled off entered into an isotope exchange either with K_2CO_3 [8] or with gaseous carbon dioxide [9].

By the carbon method, 1 ml of water at 100° exchanged in 20 minutes with 50 mg K_2CO_3. Carbon dioxide was then obtained from the above potassium carbonate as a result of the action of orthophosphoric acid; the isotope consistency of the oxygen of the carbon dioxide was determined in the mass spectrometer by the relationship of the masses 46 and 44.

In the case of isotope equilibration of gaseous carbon dioxide with water, 0.5 ml of water for analysis was withdrawn for every measurement and in the period of 5 hours (on shaking) the oxygen of the water entered into an isotope exchange with a determined quantity of CO_2. The carbon dioxide was then subjected to an isotope analysis in the mass-spectrometer. The figures given in Tables 1 and 2 denote the excess concentration of O^{18} in the analysis test tubes in comparison with the natural CO_2.

Results of the first series of experiments, in which seven and eight-day-old plants were used, are given in Table 1.

From the data in Table 1 we can see that 30 minutes after immersion of the roots of the plants in the food solution with heavy-oxygen-containing water, the concentration of O^{18} in the water of the cells of the roots approached an amount which was practically constant during the following 24 hours (about 60% of the concentration of O^{18} in the external food solution).

In the stem the maximum concentration of H_2O^{18} was approached noticeably later, but this amount definitely exceeded the corresponding amount in the roots and approached the concentration of H_2O^{18} in the external food solution.

The maximum saturation of heavy-oxygen-containing water in the leaves approached the maximum still later, but the concentration of H_2O^{18} in the leaves at the end of the experiment was closer to the level of that in the roots than the level in the stems.

TABLE 2

Assimilation of the H_2O^{18} into the Various Organs of 15-Day-Old Plants of Phaseolus vulgaris*

Expt. No.	Exposure	Atom % of excess O^{18} in the water					
		root	stem		leaf		
			lower half	upper half	primary	first trilobe	second trilobe
1	15 min	0.64	—	—	—	—	—
2	30 min	0.58	0.63	0.27	0.01	0.01	—
3	1 hour, 15 min	0.66	0.87	0.65	0.05	0.02	0.02
4	3 hours	0.56	0.85	0.63	—	0.03	0.02
5	6 hours	0.53	1.01	0.99	0.43	0.24	0.16
6	24 hours	0.52	0.79	0.78	0.31	0.33	0.37

* Concentration of H_2O^{18} in the food solution equaled 1.12 atom % of the excess of O^{18}.

Plants exposed 12 and 24 hours were found to have a somewhat reduced concentration of O^{18} in the roots and stems. It is possible that this took place because, as a result of the successive immersion of plants of all series in the same food solution with H_2O^{18}, a change in the dilution resulted from the migration of water from the roots.

The second series of experiments was carried out with more developed plants. The establishment of the experiment was similar to the primary experiment with the only differences being: a) the first isotope analysis of the water of the roots was made 15 minutes after they were immersed in the food solution; b) the stems were differentiated into lower and upper halves; and c) water from the different halves was analyzed separately. Here, the basic results of the first experiments were confirmed (Table 2).

As seen from the data given in Table 2, the maximum concentration of H_2O^{18} in the roots was already reached 15 minutes after immersion of the roots in the food solution and was held practically constant at this level for the entire period following.

The concentration of H_2O^{18} in the upper and lower halves of the stem approaches the same level in the final result; however, up to the time of approaching this level, the lower half of the stem was noticeably leading the upper half in the concentration of H_2O^{18}.

In the leaves the same tendency for exchange of the ordinary water with the heavy-oxygen-containing water was found, though here it also progresses more sluggishly than for the plants of the first series of experiments.

Two works have immediate relation to the question we are considering. These investigations are connected with the assimilation of isotopes of hydrogen into plant cells. Cline [1] working with tritium oxide showed that on increasing the time bean plants are kept on the food solution containing tritium oxide, the cells of the plant (leaves and stems) are enriched with tritium oxide to 45 - 65 % of the level of the external solution. Considering this effect, Cline expressed doubt that it could be caused by the presence of colloid-associated water in the plant cells. In connection with the second possible reason, the author used different rates of absorption and translocation of molecules of tritium oxide and ordinary water, that is, the phenomenon of the isotope effect. Because the isotopes of hydrogen (tritium and deuterium), as already noted, are in mass two-three times different from the ordinary isotope of hydrogen, the suppositions of the author can be essentially accepted.

In the experiments with Elodea canadensis, similar data were obtained by Kutyurin [2]. The author in his experiments used a food solution enriched with deuterium oxide. Although the time of approach to equilibrium

in Kutyurin's experiments was definitely shorter, nonetheless the normal tendency of tritium oxide and deuterium assimilated into the cell was similar. According to the data of the referred to author, the variation in pH, environment, temperature, and conditions of absorption cannot essentially change the concentration of deuterium oxide in the plant cells.

The first experiments we established on the assimilation of H_2O^{18} into the plant cell showed the same regularity, that is, the absence of equilibrium (in the approach to equilibrium) between the concentration of O^{18} in the external food solution and the internal cell water, approximately in the same relations as with T and D.

It appears that this made it possible to conclude that the effect of irregular distribution of heavy water is not produced by the isotope effect, since there is little likelihood for an isotope effect for tritium and deuterium, which are abruptly distinguished by the mass from the normal isotope of hydrogen, and because O^{18} and O^{16}, which are not definitely distinguishable, coincided.

However, such a conclusion would also be an insufficient basis for the following reason. If it is suggested that the penetration of water through all the membranes takes place without its preliminary disassociation, but in the form of the entire molecule, then it seems that the difference in the mass of THO, H_2O^{18}, and DHO is so insignificant that the proposition of there being a coincidence of isotope effect stands as completely realistic.

The considerations given above suggest that the absence of an equality in the concentration of H_2O^{18} between the food solution and the cellular water, as shown by us, by itself still cannot finally decide which is the reason for its effect. Supplementary knowledge is needed for this.

The answer was obtained in the following experiments in which a differential isotope analysis of the water of the various organs was made (Tables 1 and 2). First of all, it seemed that in spite of the fact that the roots were entirely immersed in the food solution with H_2O^{18} and kept there for a very long time, the concentration of O^{18} in the roots did not approach the level of its concentration in the food solution. On the other hand, the concentration of heavy-oxygen-containing water in stems seemed significantly higher than in the roots, where, in several experiments (Experiment 3 in Table 1 and Experiment 5 in Table 2), the concentration of H_2O^{18} in the stems practically reached the level of its concentration in the food solution.

It seems to us that the results of these experiments already more definitely suggest that the inequality of the distribution of H_2O^{18} between the food solution and the organs of the plant (roots, leaves), as differing in our experiments, do not suggest the results of the isotope effect inasmuch as the stem, which is more removed in space from the food solution, has a significantly higher concentration of H_2O^{18} than the roots, which were placed in the solution. The concentration of heavy-oxygen-containing water in the stem approaches the concentration in the food solution.

It appears to us that the above data suggest that the roots and leaves have an equal and significant quantity of water (about 40%) that is not readily exchangeable with the H_2O^{18} entering from without. In contrast to the roots and leaves, in the stem the relationship of the not-readily-exchangeable water to the normal concentration of water, it appears, is reduced to the minimum. The results of the experiment in which the plants were grown from seed on a food solution with a known concentration of H_2O^{18} also suggest the use of such an idea.

The first such experiment was established by us with seeds of sunflower, Helianthus annus [10]. The isotope analysis showed that the concentration of H_2O^{18} in the water of the cells of these plants approaches the concentration of heavy hydrogen in the solution on which the plants were grown. Similar results were also obtained with Phaseolus aureus (Table 3).

A somewhat lower concentration of H_2O^{18} in the plant cells in comparison with the food solution is probably explained by the fact that the initial seeds contained normal water which could dilute to some degree the H_2O^{18} assimilated into the plant. Besides this, the dilution could also come about as a result of biosynthesis of water in the plant cells as a result of fixation of atmospheric O_2 in the process of respiration, as we showed earlier using O^{18} [11]. All this, of course, does not exclude the possibility of an insignificant fractionation of heavy-oxygen-containing water and ordinary water by the plant cells.

The high percent of exchange of water in the stem, in comparison with the leaves and roots, and also its comparatively rapid assimilation, as established in our experiments, demands explanation. It seems to us that for this, two important hypotheses can be attractive: either it is necessary to suppose that a very intensive

exchange of water takes place from the xylem in a radial direction or that, differing from the classical idea about the xylem as the only path for the translocation of water in the stem, actually the translocation also takes place in the parenchyma cells of the stem.

TABLE 3

The Concentration of Heavy-Oxygen-Containing Water in the Plant Cells of Phaseolus aureus Grown on a Food Solution with H_2O^{18}

Expt. No.	Variant	Age of the plants, days	Height of the plants, cm	Dry weight per plant (without roots), mg*	Atom% of O^{18}
1	Plants grown in the light (2,500 meter-candles)	9	7	56	1.23
2	Plants grown in the dark	7	22	400	1.12
3	Concentration of O^{18} in the food solution	—	—	—	1.40

* Average weight of one seed approximately 50 g.

The results of our experiments suggest the use of the second idea. Actually, in connection with the data given in Table 2 at any rate, after every 15 minutes in the roots about 60% of the water changed afresh with the water entering from without. The same amount of water must leave the roots in any period as is moved upward by the stem. Considering the relative weight of the conducting fasicles to the normal mass of cells of the stem and root, it can be proved that in less than 30 minutes the entire xylem will be filled with this " transition" water (if it is assumed that the translocation of water takes place only in the xylem). Consequently up to this time various possiblities exist for the exchange of H_2O^{18} held in the xylem with the parenchyma cells of the upper and lower halves of the stem and the differences between these parts of the stem in the concentration of H_2O^{18} after 2-3 hours practically cannot be detected.

In reality, as we can see from Table 2, the difference exists and is maintained for a fairly long period of time, after which it dwindles to nothing. These observations are more readily explained if it is imagined that the parenchyma cells of the stem also serve in the transport of water from the roots in the direction of the leaves. It is understood, however, that the intensive exchange of water in the cells of the stem must also have a place in this case.

SUMMARY

1. On keeping 7-15 day-old kidney bean plants on a food solution containing H_2O^{18}, the ordinary water of the plant cells is gradually replaced with heavy-oxygen-containing water.

2. However, even on long exposure, in which an equilibrium between the concentration of H_2O^{18} in the plant cells and in the food solution is approached, an equality in the concentration of heavy-oxygen-containing water in them is not approached. The concentration of H_2O^{18} in the cells of the root and leaf makes up only about 60% of its concentration in the water of the food solution.

3. In the roots a very rapid approach of the moving equilibrium is observed in the 15 minutes after immersion in the food solution with H_2O^{18}.

4. In the stems the concentration of H_2O^{18} approaches equlibrium at a significantly higher level than in the roots and leaves and at a level approaching the concentration in the food solution.

5. On growing plants from seed on a food solution containing heavy-oxygen-containing water, the concentration of O^{18} in the water of the cells nearly approaches the concentration of H_2O^{18} in the food solution.

6. On the basis of all this, the authors come to the conclusion that the observed absence of an equality in the concentration of heavy water in the plant organs and in the food medium suggests that there is no isotope effect, but that this absence comes about as a result of the presence of not-readily-exchangeable water in the plant cells.

7. It appears that the plant organs with intense metabolism (root, leaf) contain significantly more water which is not readily exchanged.

8. As a result of the analysis of the data obtained, the possible paths of movement of water from the roots to the aerial organs of grassy plants are considered in the article.

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THE EFFECT OF PHOSPHORUS ON LIGHT AND DARK REACTIONS OF PHOTOSYNTHESIS IN SCENEDESMUS QUADRICAUDA

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Among the nutrient mineral elements, phosphorus occupies a unique position. Phosphorus-containing proteins, nucleoproteins, play a very important role in the vital activities of organisms. Phosphorus is a component part of many intermediate metabolites and also of the prosthetic groups of a number of enzymes involved in respiration, fermentation, carbohydrate metabolism, and numerous other intracellular conversions. A whole series of processes occur at the expense of phosphate-bond energy in living organisms.

In spite of the important role of phosphorus in metabolism, it was for a long time unknown whether this element was involved in photosynthesis, and only in the last 12-15 years was it definitely shown to be so involved.

Results of experiments with extended growth of terrestrial plants under varying phosphate regimes were highly variable. To a large extent this is explained by differences in the rate of development of plants exposed to different levels of phosphorus as well as to differences in the physiological status of plants of the same age [1]. More clear-cut results showing that phosphorus exerts a stimulatory effect were obtained in short-term experiments; plants which were grown in identical nutrient environments and which were as a result identical in their physiological condition were given phosphorus through the leaves either by injection or by surface application [2, 3].

Clear evidence that phosphorus causes an increase in photosynthetic activity is contained in a number of studies carried out in recent years with aquatic plants [4, 5]. This was also observed in our experiments with certain algae in which phosphorus was added either to pond water or tap water [6, 7]. The effect was the same whether plants were exposed to the given solutions 3-15 days or 1-2 hours.

The increase in assimilatory activity of aquatic plants which was observed in these studies was not due to an increazed assimilating surface, since the rate of photosynthesis was based on unit weight, unit area, or on a single individual in the case of one-celled algae. It could not be accounted for, moreover, by changes in chlorophyll content, as shown by results of numerous short-term experiments carried out both by us and by other investigators in which the stimulatory effect of phosphorus on photosynthesis was unaccompanied by alteration of chlorophyll content. An experiment performed in our laboratory in 1953 with Scenedesmus quadricauda after the alga had been grown two weeks at various phosphorus levels confirmed this (Table 1).

In making an approach to a physiological analysis of the effect of phosphorus on photosynthesis, it would seem important to first determine which reactions, light or dark, are affected, and this was the purpose of this study.

In the solution of this problem the effect of phosphorus on photosynthetic rate at various light intensities was studied. It is known that at low light intensity the rate is limited by the light reactions and at high intensity by the dark reactions. By studying the effect of a given factor on photosynthesis at various intensities and establishing that region in which the effect is manifested, it is possible to determine which reactions are involved [8-10].

The study described below was performed in 1956 with the one-celled protococcal alga Scenedesmus quadricauda.

TABLE 1

Rate of Photosynthesis and Chlorophyll Content of Scenedesmus quadricauda in Relation to Phosphate Nutrition

Phosphorus in mg/l	Rate of photosynthesis, O_2 in μg per million cells per hr.	Chlorophyll content in μg per million cells	Assimilation No. (O_2 per unit chlorophyll per hr)
5.7	12.1	1.67	7.25
1.14	6.5	1.62	4.02
0.57	4.4	1.53	2.88

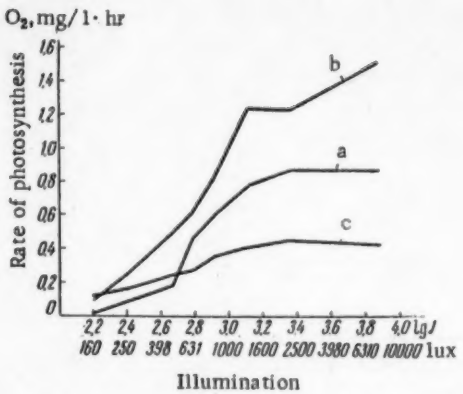

Fig. 1. Dependence of rate of photosynthesis of Scenedesmus quadricauda on illumination. Experiments performed: a) Aug. 2; b) Aug. 9-10; c) Aug. 13-14.

The plants were cultured on Knop's solution, diluted tenfold, prepared with tap water which had been boiled 20-25 minutes. The culture was distributed among 500-600 flasks provided with cotton plugs. The nutrient solution was changed every 10-15 days.

The plants selected for determination of photosynthetic rates were placed in darkness the morning of the experiment. Preliminary experiments had indicated that under these conditions rates fluctuated less in the course of a day than when the culture had remained in the light. For each determination equal aliquots were removed from the culture flask for the various experimental treatments. In order to separate the algae an aliquot was centrifuged 10 minutes at 2000 rpm. The supernate was decanted and the residual algae resuspended in water. The algal suspension was then shaden up and divided between two flasks. To one of the flasks was added NaH_2PO_4 at 2 mg P per liter; the other flask served as a control. The algal suspension in each flask was further divided and poured into two vials which were exposed to light for determination of photosynthetic activity. For this determination tap water which had stood in open vessels for two or three days was used.

In order to maintain a more or less constant temperature the vials containing the plants and the two controls with water were placed on the bottom of a crystallizing basin supplied with running water. The experiments were performed in a dark room, the plants being illuminated from above with incandescent lamps.

The rate of photosynthesis (and the rate of respiration in certain experiments) was determined by the difference in the amount of dissolved oxygen in the experimental vials before and after exposure. Oxygen was determined by Winkler's method. The experiments were carried out with suspensions containing 200-300 million cells per liter. The photosynthetic rate (observed) and the respiration are expressed in μg oxygen per million cells per hour. The number of cells was determined in a counting chamber under the microscope. In certain experiments the rate of photosynthesis was expressed in mg oxygen per liter culture suspension per hour. Each determination was made in duplicate.

Before doing experiments on the effect of phosphorus, it was necessary to determine the regions of high and low light intensity for a given culture. These depend on plant species, on the condition of the plant, and on growth conditions, and must therefore be experimentally determined for each subject. If changes in light intensity are accompanied by corresponding changes in photosynthetic rate, this is the region of weak intensity; if, however, changes in light intensity have no effect, then this is the region of high intensity. Accordingly, a series of experiments to determine the dependence of photosynthesis in Scenedesmus quadricauda on light intensity was performed. Various intensities were obtained with 25, 40, 150, 200 and 300 watt incandescent lamps placed at varying distances from the plants.

TABLE 2

The Temperature Coefficient (Q_{10}) for Photosynthesis at Various Light Intensities in Scenedesmus quadricauda

Light intensity, in lux	Temp. deg C	Treatment -P: Rate of photosynthesis Mg O^2 per liter algal, determined	Treatment -P: Rate of photosynthesis Mg O^2 per liter algal, average	Treatment -P: Q_{10}	Tretatment + P: Rate of photosynthesis Mg O^2 per liter algal, determined	Tretatment + P: Rate of photosynthesis Mg O^2 per liter algal, average	Tretatment + P: Q_{10}
5000	11	0.48 0.70	0.59	2,52	1.29 1.18	1.23	1.51
	21	1.48 1.50	1.49		1.87 1.86	1.86	
600	11	1.44 1.08	1.26	0,88	1.42 1.42	1.42	1.00
	21	1.11 1.14	1.11		1.36 1.50	1.43	
160	11	0.90 0.63	0.76	1.00	1.03 1.11	1.07	1.00
	21.	0.75 0.76	0.75		1.05 1.09	1.07	

TABLE 3

The Effect of Phosphorus on the Photosynthetic Rate in Scenedesmus quadricauda at Various Light Intensities

Light intensity in lux	Expt. 3 (Aug. 9-10) μg O_2/million cells/hr: −P, replicate value	−P, average	+P, replicate value	+P, average	+P in % of −P	Expt. 4 (Aug. 13-14) μg O_2/million cells/hr: −P, replicate value	−P, average	+P, replicate value	+P, average	+P in % of −P
160	0.25 0.31	0.28	0.25 0.28	0.27	96	0.33 0.33	0.33	0.33 0.33	0.33	100
250	0.65 0.65	0.65	0.87 0.90	0.88	135	0.54 0.51	0.52	0.66 0.75	0.70	135
440	1.52 1.49	1.50	1.74 1.74	1.74	116	0.75 0.75	0.75	0.96 0.93	0.94	125
600	1.86 1.92	1.89	2.76 2.76	2.76	146	0.84 0.87	0.85	1.26 1.29	1.27	149
800	2.48 2.51	2.49	3.35 3.35	3.35	134	1.08 1.08	1.08	1.74 1.74	1.74	161
1250	3.84 3.87	3.85	4.71 4.72	4.71	122	1.29 1.26	1.27	1.80 1.92	1.86	146
2220	3.84 3.81	3.83	5.15 5.15	5.15	134	1.50 1.29	1.39	2.22 2.16	2.19	158
7520	4.77 4.68	4.72	6.04 6.08	6.06	128	1.29 1.29	1.29	2.13 2.13	2.13	165

From the results presented in Figure 1 it can be seen that the region of weak intensity for the given plants extended up to approximately 800 lux (even higher in some case), and the region of high intensity extended from 2000 lux.

TABLE 4

The Effect of Phosphorus on the Photosynthetic Rate in Scenedesmus quadricauda at Various Light Intensities

Light intensity, in lux	Expt. 5 (Aug. 16-17) O_2 mg/liter algal suspension/hr					Expt. 6 (Aug. 22-23) O_2 mg/liter algal suspension/hr				
	−P		+P		+P in % of −P	−P		+P		+P in % of −P
	replicate value	average	replicate value	average		replicate value	average	replicate value	average	
50	−0.06 −0.07	−0.07	−0.02 −0.06	−0.04	—*	0.18 0.19	0.18	0.29 0.29	0.29	161
100	−0.03 −0.03	−0.03	0.015 0.015	0.015	—*	0.30 0.30	0.30	0.38 0.35	0.36	120
160	0.04 0.07	0.05	0.08 0.10	0.09	—*	— —	—	— —	—	—
250	0.24 0.17	0.20	0.25 0.28	0.26	130	— —	—	— —	—	—
440	0.32 0.31	0.31	0.49 0.52	0.50	161	0.36 0.27	0.31	0.48 0.49	0.48	155
600	0.42 0.42	0.42	0.80 0.82	0.81	193	0.45 0.44	0.44	0.72 0.71	0.71	161
800	0.48 0.51	0.49	0.80 0.88	0.84	171	— —	—	— —	—	—
2220	— —	—	— —	—	—	0.59 0.53	0.56	0.81 0.92	0.86	153
5000	— —	—	— —	—	—	0.54 0.60	0.57	0.95 0.84	0.89	156
7520	— —	—	— —	—	—	0.60 0.54	0.57	0.92 0.95	0.93	163

* For negative or low values of the assimilation rate the relative values were not calculated.

A further check on these determinations was provided by the temperature coefficients (Q_{10}) at these light intensities. The Q_{10} for photosynthesis should be close to one at low intensities where the rate is limited by the photochemical reactions, and higher at higher intensities where the rate is limited by dark reactions. Accordingly, determinations of Q_{10} over the temperature range 10-11° to 21-22° were made at various light intensities.

For these determinations we utilized a method described in a paper by Mikhailova [11].

Data summarized in Table 2 show that at intensities of 160 and 600 lux the Q_{10} was equal to one, or nearly so, both in the presence and the absence of phosphorus; at 5000 lux it was 1.51 and 2.52 respectively.

Having determined the relationship of photosynthesis in Scenedesmus quadricauda to light intensity, as well as the regions of high and low intensity under the experimental conditions employed, we turned to the principal problem of the study, the determination of the effect of phosphorus on photosynthesis at different light intensities. Six experiments were performed. Photosynthesis was measured at intensities ranging from 50 to 7520 lux. In each experiment three or four determinations were made, both in the low-intensity range and the high-intensity range. A culture was used which had first been grown for 13-15 days on Knop's nutrient solution, diluted tenfold, and then for several days on a fresh nutrient solution with phosphorus omitted.

The exposure period was one hour. In a few experiments at low intensities it was increased to 2 hours because of the small amounts of oxygen evolved.

Results of a few experiments, which are sufficient to illustrate the patterns obtained, are presented in Tables 3 and 4.

From the data summarized in Tables 3 and 4 it is clear that at both high intensities (2220-7520 lux) and low intensities (50-600 lux) the rate of photosynthesis is increased 20-60% by the addition of phosphorus, and even more in some cases.

The same results were obtained by Fattakhova in 1953 in his thesis work. Addition of phosphorus caused an increase in the rate of photosynthesis of Scenedesmus quadricauda of 15-40%, both at high and low intensities.

A stimulating effect of phosphorus on photosynthesis at various light intensities was also demonstrated in experiments in which the temperature coefficient was determined. In Table 2 are presented results of Q_{10} determinations in the presence as well as in the absence of phosphorus. At all light intensities and at both temperatures, the addition of phosphorus resulted in an increase of 20-40% in the photosynthetic rate.

The stimulatory effect of phosphorus observed at both high and low intensities indicates that both the dark and the light reactions are being affected.

A few experiments were performed to determine the effect of phosphorus on respiration of the alga. The increase in assimilation rate observed could be due not only to an increased rate of photosynthesis but also to a decreased rate of respiration. The experiments showed that the respiratory rate of the plants studied was comparatively small. Moreover, the addition of phosphorus had either no effect or a somewhat stimulatory effect on respiration, a fact of particular importance in the experiments performed at low intensities. A correction for respiration would not, therefore, alter the patterns observed, and would even in some cases cause them to be more sharply defined.

Studies of the past twenty years have provided much new information of value in an explanation of the mechanism of the effect of phosphorus on photosynthesis. Upon discovery of the role of high-energy phosphorus compounds in chemosynthesis [12, 13] during the 1940's, the question of a possible participation of these compounds in energy conversions in photosynthesis arose. The hypothesis was advanced that all the energy of the quanta absorbed during photosynthesis was first converted into phosphate-bond energy which was further utilized in the dark reactions [14]. This point of view met opposition from many quarters by workers who suggested that a more diversified utilization of light energy in photosynthesis was possible [15-17].

While the formation of energy-rich phosphate bonds should not be considered the unique result of the photochemical reaction, it is true, as demonstrated in recent years, that photosynthesis is closely involved with phosphate conversions, and many phosphorylated compounds play a part in individual reactions of photosynthesis [18].

Calvin [19] and other workers have shown that phosphorus is present in many photosynthetic intermediates, that the process of fixation and reduction of CO_2 is related at many points to phosphorus-containing compounds—ATP, pyridine nucleotides, and others. The ability of chloroplasts to carry on reductive carboxylation in the light in the presence of pyridine nucleotides was demonstrated by Vishniac and Ochoa [20, 21].

That there is a connection between conversions of phosphorus compounds and photosynthesis was also shown by the observations of many workers [22, 23] of increases in the light of the acid-soluble organic phosphorus fraction. A number of studies have demonstrated the formation of energy-rich phosphorylated compounds during photosynthesis [24-27].

Our data and those of other workers [4, 5] indicating a stimulation of the dark reactions by phosphorus are in complete agreement with contemporary views on the participation of phosphorus in a number of enzymatic reactions of photosynthesis. The stimulatory effect of phosphorus under conditions where light reactions are limiting is more difficult to explain.

At the present time the light reaction is considered to be the reaction of chlorophyll photoreduction. As the studies of Krasnovskii have shown, photoreduced chlorophyll may transfer hydrogen to oxidized forms of the prosthetic groups of dehydrogenases — pyridine nucleotides — which then participate in reactions of CO_2 reduction.

Thus, phosphorus does not participate directly in the photochemical reaction as it is conceived at the present time. But hydrogen transfer from the reduced form of chlorophyll to pyridine nucleotides is closely related to the photoreduction of chlorophyll. Since the transfer of hydrogen occurs at the expense of phosphorus-containing compounds, a deficiency of the latter would probably inhibit chlorophyll photoreduction.

The stimulatory effect of phosphorus on photosynthesis which was clearly manifested in our experiments may be due, in our opinion, to certain physiological peculiarities of the experimental subject [30]. Specifically, it is conceivable that in plants with low respiratory activity and, consequently, weak oxidative phosphorylation, the high-energy phosphorus compounds formed during photosynthesis assume great importance in metabolism and CO_2 assimilation.

From the evidence presented it may be concluded that phosphorus exerted an effect on both the dark and light reactions of photosynthesis in Scenedesmus quadricauda.

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SYNTHESIS OF AMINO ACIDS IN ROOTS OF THE POTATO PLANT AT VARIOUS PERIODS OF THE DAY AND UNDER VARIOUS PHOTOPERIODIC CONDITIONS

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In recent years significant progress in the study of the root system as a metabolic unit in plants has been made [1]. Specifically, the studies of a number of workers [2-6] have established that amino acid synthesis occurs in roots.

This synthetic function of roots is of particular interest in the study of the role of the root system in plant growth and development. More and more information is being obtained which shows that plant growth proceeds successfully only when the root system is functioning normally [7-10]. Sabinin [11] explained this in terms of synthesis in the roots of physiologically active compounds of the phytohormone type, these being, apparently, nucleic acid derivatives. The participation of the roots in developmental processes was demonstrated by Chailakhyan [12, 13] in experiments in which Rudbeckia plants deprived of roots were shown to be incapable of a photoperiodic response, this being correlated with the fact that certain metabolites from the roots ceased to appear in the stem growing points. The actual relationship between the synthetic activity of the roots and development and growth has, however, received insufficient attention. One of the possible methods of study of this relationship is a comparison of the synthetic processes occurring in the roots with growth and development processes in the plant as a whole.

In our investigations, carried out in 1956-1957, diurnal changes in the amino acid composition of the sap were studied in connection with the diurnal growth periodicity of the tubers and stems. In addition, the amino acid composition of the sap of leaves, stems and tubers of plants grown at various photoperiods was studied in connection with the photoperiodic response.

METHODS

Experiments were performed using the varieties Lorkh and Épron. Plants were grown on gray podzolic soil in the field under long (natural) and short (ten-hour) days.

To obtain sap, the stems were cut off at 3-4 cm. A triangular piece of filter paper was placed on the cut surface with the pointed end leading into a sap receiver (Fig. 1, a). The sap exudate passed through the filter paper into the receiver without loss. When the soil moisture was at 70% of field capacity, it was possible to obtain 150 ml of sap in a twenty-four hour period by this method. In order to prevent concentration of the sap by evaporation, the stem and the receiver were enclosed in a glass bell jar lined with moist filter paper. The damp chamber was covered to keep out light.

For extended collections of sap from plants with ribbed stems we also used the following method with success (Fig. 1, b). The stem was enclosed in melted paraffin. A cylinder of thick paper served as a form. The paraffin covering formed in cooling closely girdled the stem. The embedded stem was then cut across and the exposed surface covered with a small glass bell jar as shown in Fig. 1, b. The sap flowed from under the bell jar through a straw which had been placed in the paraffin at the time it was poured. Every three-five days the

bell jar was removed and the stem was severed at a lower position. With this method we studied the exudates of a single stem over a period of 10-15 days. The cut surface of the stem was not kept sterile, but toluene was routinely added to the sap receiver.

In the study of diurnal changes in sap composition, periodic collections were made either with a fraction collector or by a periodic change of sap receivers every 1-2-4 hours. In the latter case the sap collected simultaneously from 20-40 stems was pooled.

In the study of amino acid content 10 ml of sap were evaporated in an enamel dish on a water bath. The dry residue was taken up in 0.2 ml of water and spotted on chromatographic paper in 0.01 ml aliquots.

Leaves, stems and tubers were fixed for 10 minutes in steam after collection; subsequently they were dried and minced. Amino acids were extracted from 400 mg of dry material with 80% ethanol for 1 hour on a water bath. The dry residue obtained by evaporation of the extract was dissolved in 0.1 ml of water and spotted on paper in 0.01 ml aliquots.

Amino acids were separated on descending chromatograms with a mixture of n-butanol, acetic acid and water (4 : 1 : 5). Unfortunately one-dimensional chromatography was not sufficient to separate amino acids with similar R_f values; in the discussion of results, therefore, we will be concerned primarily with those amino acids which were clearly separated on our chromatograms.

The total amino acid content was determined colorimetrically. To 5 ml of sap was added 0.5 ml of a 5% solution of ninhydrin in MacIlvaine's buffer at pH 7, and the resulting mixture was heated on a boiling water bath for 15 minutes. The intensity of the color which had developed was determined in a colorimeter. Amino acid content was calculated on the basis of a calibration curve obtained with a mixture of glycocoll, serine, alanine and leucine.

EXPERIMENTAL RESULTS

Diurnal changes in amino acid composition of the sap were followed with five collections made at various stages of growth. The chromatograms obtained showed that the amount and composition of amino acids changed in a regular fashion in the course of twenty-four hours. The diurnal change in amino acid composition of sap collected from the variety Lorkh (July 19-20, 1956) is shown in Fig. 2.

In sap collected from 12 noon to 3 P.M. (Fig. 2, I) about ten amino acids were identified – leucine, phenylalanine, tyrosine, alanine, histidine and amino acids of the aspartic acid, glycocoll, glutamic acid, serine group. In sap collected from 5 P.M. to 7 P.M. (Fig. 2, II), the amounts of individual amino acids had decreased and phenylalanine and tyrosine had disappeared. In sap collected at night – from 11 P.M. to 3 A.M. (Fig. 2, III), only two amino acids were found – alanine and some unidentified amino acid x. According to its position on the chromatogram it belongs to the aspartic acid, glutamic acid, glycocoll, serine group. In the sample collected from 5 to 9 A.M. (Fig. 2, IV), the amounts of amino acids present had once more increased. Leucine and

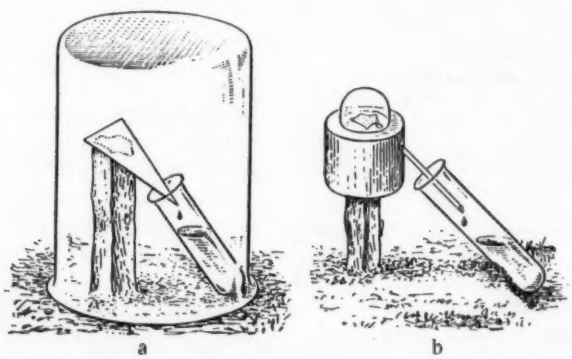

Fig. 1. Apparatus for sap collection in potato (explanation in text).

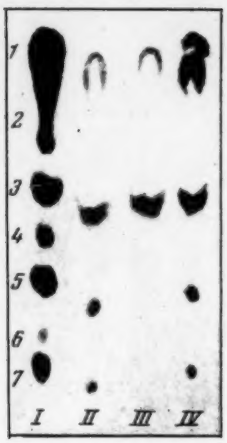

Fig. 2.

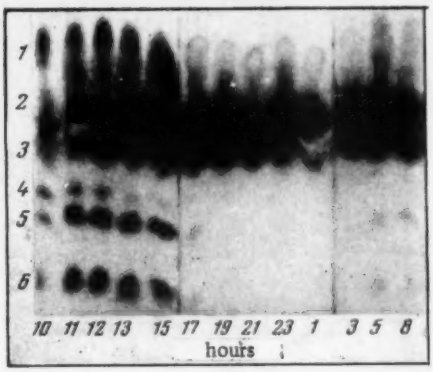

Fig. 3.

Fig.2. Amino acids in the sap of Lorkh potato collected at various times of the day. I) From 12 noon to 3 P.M; II) from 5 to 7 P.M; III) from 11 P.M to 3 A.M.; IV) from 5 to 9 A.M; 1) histidine; 2) aspartic acid, glutamic acid, glycocoll, serine and others; 3) alanine; 4) tyrosine; 5) valine, methionine; 6) phenylalanine; 7) leucine.

Fig. 3. Amino acids in the sap of Épron potato collected at various hours of the day. 1,2) Unidentified; 3) alanine; 4) tyrosine; 5) valine; 6) leucine. Horizontal figures) hours of the day.

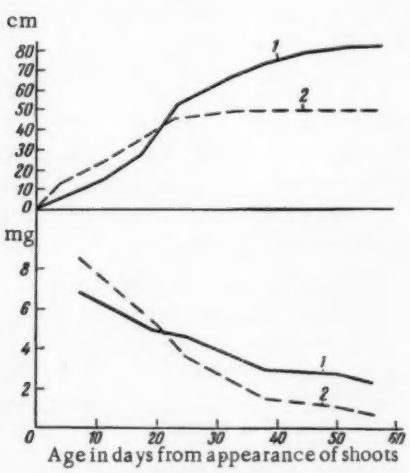

Fig. 4. Relation between stem growth (height in cm – upper curves) and amino acid content of the sap (in mg per 100 ml – lower curves) in variety Lorkh.
1) On long days; 2) on short days.

valine had appeared, but phenylalanine and typrosine were still absent.

Similar diurnal changes in amino acid composition of the sap were noted in other cases. In Fig. 3 are shown the diurnal changes in the amino acid composition of sap collected on June 29-30, 1956, from the variety Épron. It is true that on this chromatogram there is no clear separation of amino acids with similar R_f values, but it may be stated with certainty that tyrosine, valine and leucine are absent in the night hours and are present in considerable amounts in the daylight hours.

On the basis of the data presented above.it may be suggested that there is a diurnal rhythm in the synthetic activity of the roots of potato; possibly this is related to a corresponding periodicity of flow of photosynthate to the roots and also to a more intensive absorptive activity of the roots in the daylight hours, as was shown for calcium and phosphorus [14]. Also of interest is the pattern of cessation of synthesis of individual amino acids in the evening hours and the resumption of their synthesis in the morning hours. At night only, the simplest amino acids are found in the sap, such as alanine, glutamic acid and aspartic acid, which are formed by amination and transamination of mono- and dicarboxylic keto-acids. In the early morning hours, more complex compounds with aliphatic chains are found (leucine, valine), and during the day, cyclic compounds formed by transamination and enzymatic conversion of other amino acids are found (histidine,

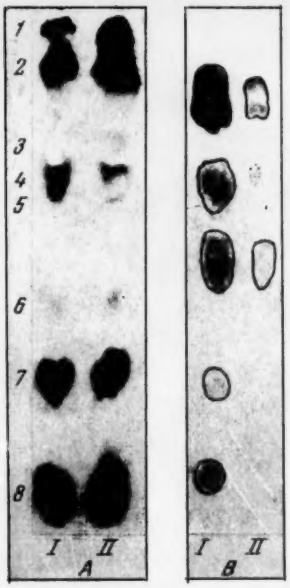

Fig. 5

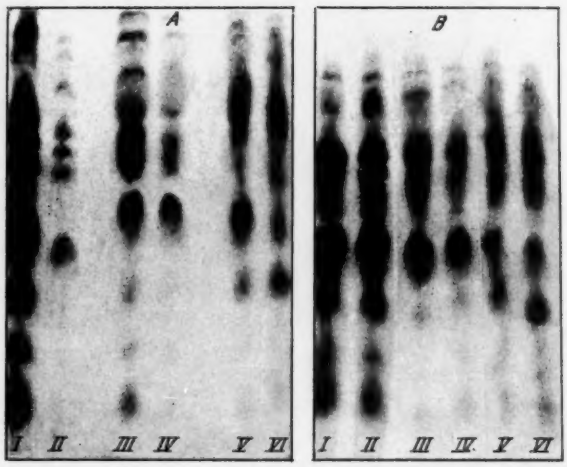

Fig. 6

Fig. 5. Amino acids in the sap of Lorkh potato on long days (I) and on short days (II). A) Four-day-old plants; B) 30-day-old plants; 1, 2, 3) histidine, aspartic acid, glutamic acid, glycocoll and others; 4) alanine; 5) unidentified; 6) tyrosine; 7) valine, methionine; 8) leucine.

Fig. 6. Content of free amino acids in organs of Lorkh potato at various photoperiods. A) 9 A.M.; B) 1 P.M. I) leaves, long day; II) leaves, short day; III) stems, long day; IV) stems, short day; V) tubers, long day; VI) tubers, short day.

tyrosine, phenylalanine). This sequence of appearance of amino acids in the sap agrees very well with data from another study [15]. These workers investigated the sequence of incorporation of N^{15} into amino acids during their synthesis by plants and found that labeled nitrogen first appears in alanine, after 5-30 minutes. After 1-2 hours it appears in dicarboxylic amino acids and after 12-36 hours — in basic and aromatic amino acids.

In an earlier study [16] we showed that there is a diurnal periodicity of growth of tubers and stems in potato under normal conditions of temperature and soil and air moisture content which is characterized by a rapid growth in the daytime and a cessation of growth at night. Thus, the diurnal periodicity of growth corresponds with the diurnal periodicity of amino acid synthesis in the roots. It may be assumed that this is not fortuitous; the effect of the root system on growth is mediated, apparently, not only through synthesis of phytohormones in the roots, as D.A. Sabinin thought, but also through synthesis of amino acids utilized in growth.

It is well known that the principal plant organ acting as a receptor of the photoperiodic stimulus is the photosynthetically active green leaf [17, 18]. It is undoubtedly true, moreover, that the plant organism responds to a photoperiod as a unit, with the participation not only of the leaf but of other organs as well. To be specific, Chailakhyan [12, 13, 19], on the basis of his experiments, has advanced the hypothesis that the root system plays an active role in the photoperiodic response.

In this connection it seemed appropriate to compare the characteristics of amino acid synthesis in potato roots at various photoperiods, using for this purpose sap analysis.

Many workers (see tables in reviews of Samygin [20] and Grebinskii [21] classify potato (Solanum tuberosum) as a long-day plant on the basis of the fact that it ordinarily does not flower on short days.

In our experiments [22] performed from 1953-1956 it was shown that on a short day potato does not flower but always forms small buds, up to 2-3 mm, which turn yellow and fall off. The formation of tubers begins from

five to seven days earlier on a short day than on a long day, and the weight of tubers formed per unit weight of green tissue is always greater on a short day. The first 10-15 days after the appearance of shoots stem growth and accumulation of leaf and stem mass is more rapid on a short day, but growth of leaves on a short day ceases 25-40 days earlier than on a long day and death occurs earlier. Because of this the variety Lorkh, which is moderately late in the central Urals, is early on a short day and by Aug. 15-25 has already ceased growth. In potato a photoperiodic aftereffect is clearly manifested. Plants which had received 10-12 successive short days after germination did not begin to flower after being removed to long days and developed as did plants which had been kept on short days throughout the growth period.

On the basis of these facts [22] we asserted that development of the cultivated potato on a short day is hastened.

In 1956-1957 we studied the changes in amino acid content of the sap with age of plants grown on long and short days. Collections of sap were made systematically every 8-10 days from the appearance of shoots to the death of the plants grown on short days. Sap exuded in the course of a twenty-hour period after cutting of the stem was collected for analyses. In this way the effect of diurnal periodicity was excluded. Observations of the dynamics of growth of the green tissues on long and short days were made by measurements of 30 selected stems in each experimental group every 5 days.

The data presented in Fig. 4 show the changes with age of amino acid content of the sap (lower graph) and of stem height (upper graph) in the variety Lorkh on long (1) and short (2) days in the 1957 experiment. In a comparison of the curves the relationship of growth rate to amino acid content of the sap becomes clearly apparent.

In young, rapidly growing plants the amino acid content reaches 9 mg per 100 ml of sap. As the plant ages this rapidly decreases and falls to 0.6 mg on the 58th day. As the graph shows, in the first days after appearance of shoots the plants on a short day grow considerably faster than the plants on a long day. But at 20 days the plants on a short day begin to lag behind the plants on a long day, and this lag increases more and more with time.

Correspondingly, the amino acid content of the sap is higher in plants on a short day during the first 18 days of the growth, and in the succeeding days the curves intersect, and the amino acid content of the sap becomes greater in plants on a long day.

Such a correspondence between changes with age in synthetic activity of roots and stem growth patterns is apparently analogous to the parallelism between the diurnal rhythm of amino acid synthesis by roots and of tuber growth noted earlier. There is no basis for the assertion that the level of synthetic activity of the roots determines the rate of growth in stems or tubers. On the contrary, the metabolic activity of the root system is determined by the growth and development status of the plant. It is quite possible, however, that the amount and kind of amino acids flowing from the roots to the growing organs exerts a substantial effect on the growth of these organs.

In Figure 5 is shown the distribution of amino acids in the sap under long (I) and short (II) days. The first chromatogram (A) shows the composition of the sap of four-day-old plants, the second (B), of 30-day-old plants. The kinds of amino acids in four-day-old plants are the same for both photoperiods. The predominant amino acids are those in the group with similar R_f values (aspartic acid, glutamic acid and others), and alanine, valine and methionine, and leucine. At 30 days, however, there is already evident a marked predominance of amino acids, both in quantity and in diversity, in plants grown on long days as compared with plants grown on short days. The study of amino acids was continued with the purpose of identifying more accurately the individual compounds. In these same plants the diurnal rhythm and age dynamics of the free amino acid composition of leaves, stems and tubers was studied in order to determine whether there are specific types of distribution of amino acids at various photoperiods in these organs. In a large number of analyses we did not observe qualitative differences in amino acid composition of leaves, stems and tubers of plants grown at different day lengths (Fig.6). Only at 9 o'clock in the morning was there found to be a larger amount of each amino acid in a long day (Fig. 6, A). Since these differences are especially large in leaves, it is possible that they are related to a later inception of photosynthesis in plants on a short day. At 1 o'clock in the afternoon, the differences in amino acid content of organs of plants on long and short days are almost completely obliterated (Fig. 6, B).

Thus, in the cultivated potato there is a more rapid synthesis of amino acids in roots under short days than under long days. This is true only during the first 15-20 days after the appearance of shoots, however; subsequently

the amount and diversity of amino acids sharply declines in plants on short days, while a relatively high level of synthetic activity by the roots is maintained on long days. This may be accounted for by a more rapid aging of the root system under short days, if it is assumed that development is hastened in the cultivated potato under these conditions.

In view of the marked qualitative and quantitative differences in amino acid composition of sap from the roots at various photoperiods, the hypothesis may be advanced that the participation of the root system in the photoperiodic response is defined by the peculiarities of the synthetic activity of the roots at various day lengths.

SUMMARY

The amino acid content in the exudate of the Lorkh and Épron potato varieties was studied during various periods of the day and under different photoperiodic conditions. A rhythmicaldiurnal variation of the amino acid content in bleeding sap was observed. A larger variety as well as larger amounts of amino acids were found in the daytime. In the night time, only alanine and some unidentified amino acids from the asparagine, glutamic acid, and glycocoll group were detected in the exudate. More complex amino acids with aliphatic chains (leucine, valine) appear in the morning and cyclic amino acids (histidine, tyrosine phenylaline) during the day.

The amino acid content in the exudate of potatoes is much greater under short day conditions in 15-20 day-old plants. However the types of amino acids do not depend on the photoperiod. In older plants the amino acid content of the exudate of plants grown under short day conditions rapidly decreases and a marked quantitative and qualitative predominance of amino acids is observed in plants grown under long day conditions.

The diurnal periodicity of tuber growth and amino acid synthesis in the roots was found to be the same. The dynamics of stem growth in ontogenesis and the age variations in the synthetic activity of the roots were also found to be identical. Periods of most intense growth of organs of the potato plant coincide with maximal content and variety of amino acids in the roots.

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BIOSYNTHESIS OF GLUTAMIC ACID AND GLUTAMINE IN PEA AND WHEAT SEEDLINGS

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It has previously been shown [1] that in plant tissue homogenates there is a rapid synthesis of glutamic acid from α-ketoglutarate; this synthesis is markedly enhanced in the presence of ammonia and becomes even more rapid with the simultaneous addition to the homogenate of ammonia and cozymase.

It is thus apparent that glutamic acid synthesis by homogenates proceeds as a reductive amination of α-ketoglutarate by ammonia. At the same time it has been shown by Shales [2] and by us [3] that in homogenates there is a rapid transamination between the added α-ketoglutarate and endogenous amino acids, primarily aspartic acid.

It was conceivable that the results obtained with homogenates might not be applicable without certain limitations to living plant tissues.

The present study was made in order to investigate the biosynthesis of glutamic acid from α-ketoglutarate in living plants.

MATERIALS AND METHODS

Experiments were carried out with 10-day-old seedlings of Moscow wheat and Early peas. Seedlings were grown on gauze disks impregnated with paraffin under natural light in tap water. In the experiments with wheat we used the entire aerial portion of the seedlings, and in experiments with peas only the upper portion of the seedling, 4-5 cm in length.

Solutions of potassium α-ketoglutarate and ammonium α-ketoglutarate (0.05M), and water in the control experiments, were introduced into various homogeneous portions of the seedlings by vacuum infiltration and by absorption into the transpiration stream. Alpha-ketoglutaric acid was synthesized by the method of Blyeza and Go [14], and after several recrystallizations was identified by melting point.

After vacuum infiltration the seedlings were dried with filter paper and left in a damp atmosphere for 30 minutes or 2 hours. In experiments involving absorption of the experimental solutions, the seedlings were cut up under distilled water and left in the experimental solutions for 30 minutes or 2 hours. At the end of the experiment the seedlings, washed with distilled water and dried with filter paper, were rapidly frozen in a Dewar flask with dry ice and subsequently ground in a mortar chilled with a dry ice - ethanol mixture. The well-ground frozen material was transferred quantitatively to a measuring flask to which distilled ethyl alcohol was added so that its final concentration was 80%. An aliquot of the alcoholic extract was evaporated to dryness in a vacuum. The dry residue was taken up in 1 ml of H_2O. The amino acids in the aqueous solution were quantitatively determined. At the end of each experiment samples of the plant material were taken for determination of dry matter content.

Amino acids were quantitatively determined according to Kretovich and Uspenskaya [5] by means of chromatography on buffered paper (Leningrad, slow) with buffered phenol and orthocresol as developing solvents. The data obtained for alanine may include not only alanine but also β-alanine and homoserine, which are found in germinating pea [6]. Glutamine was quantitatively determined after separation in orthocresol according to Kretovich and Uspenskaya [7].

TABLE 1

Amino Acid Content of the Upper Portions of Pea Seedlings into Which Have Been Introduced Potassium α-Ketoglutarate (K-KG) and Ammonium α-Ketoglutarate (NH_4-KG)

Amino acids	30 min exposure					2 hr exposure				
	NH_4-KG	K-KG	H_2O	Difference in μM compared with the water control		NH_4-KG	K-KG	H_2O	Difference in μM compared with the water control	
				NH_4-KG	K-KG				NH_4-KG	K-KG
Expt. 1. Vacuum infiltration of the upper portion of the seedlings										
Glutamic acid	12.15	11.75	8.25	+26.5	+23.8	13.35	11.55	9.60	+25.5	+13.3
Glutamine	17.79	5.72	7.50	+70.5	−12.2	21.63	3.65	7.92	+94.0	−29.2
Aspartic acid	5.58	6.25	6.10	−3.94	+1.13	4.72	5.17	4.50	+1.67	+5.07
Serine	6.45	5.70	6.75	−2.86	−10.0	7.20	7.20	6.60	+5.7	+5.7
Alanine	26.13	26.55	26.13	0	+4.7	27.84	23.10	28.26	−4.7	−58.0
Expt. 2. Transport into the upper portion of the seedlings										
Glutamic acid	13.20	18.60	9.15	+27.5	+64.3	21.45	32.17	5.55	+108.1	+181.1
Glutamine	11.80	3.60	6.00	+39.7	−16.4	34.20	2.20	5.80	+194.5	−24.6
Aspartic acid	5.40	6.30	6.15	−5.68	+1.13	5.70	6.45	4.95	+5.68	+11.3
Serine	4.05	3.90	4.05	0	−1.43	5.70	5.70	6.00	−2.86	−2.86
Alanine	14.6	14.0	15.00	−4.5	−11.25	17.80	14.00	15.80	+22.5	−20.2

RESULTS AND DISCUSSION

Results of amino acid determination in pea seedlings into which ammonium and potassium salts of α-ketoglutaric acid were introduced are presented in Table 1.

The data of Table 1 (Expt. 1) show that with introduction of salts of α-ketoglutaric acid into pea seedlings there was synthesis of glutamic acid. The synthesis was accompanied by a marked disappearance of glutamine, which was in this case the chief source of amino groups for glutamic acid synthesis by transamination to α-ketoglutarate. It should be noted that on all the chromatograms it was clearly evident that introduction of potassium α-ketoglutarate into seedlings induced a definite decrease in asparagine, which was also utilized in transamination to α-ketoglutarate. With respect to aspartic acid, which in homogenates is primarily involved in transaminations with α-ketoglutarate, in living tissues of pea seedlings into which potassium α-ketoglutarate had been introduced it was even synthesized to a certain extent. Apparently in this case it was formed as a result of conversion of a small portion of α-ketoglutarate to oxaloacetic acid and subsequent transamination with amino acids present in the tissues.

With introduction of ammonium α-ketoglutarate into the seedlings, there is, in addition to glutamic acid synthesis, an extremely rapid synthesis of glutamine. In experiments involving absorption of ammonium α-ketoglutarate, there is, therefore, a reduction in the amount of glutamic acid in comparison with cases in which potassium α-ketoglutarate was used. The introduction of the ammonium ion into tissues, by inducing a rapid synthesis of glutamine catalyzed by glutamine synthetase, "draws off" a significant portion of the glutamic acid which is being formed by transamination as well as by direct amination of α-ketoglutarate. Results of experiments performed with wheat seedlings are presented in Table 2.

Inspection of the data of Table 2 clearly shows that changes in amino acid content occurring in wheat seedlings are of the same type as those occurring in pea seedlings, although the absolute values differ. Introduction of α-ketoglutarate into the tissues also induced glutamic acid synthesis. The increase in glutamic acid

TABLE 2

Amino Acid Content of Wheat Seedlings into Which Have Been Introduced Potassium α-Ketoglutarate (K-KG) and Ammonium α-Ketoglutarate (NH_4-KG)

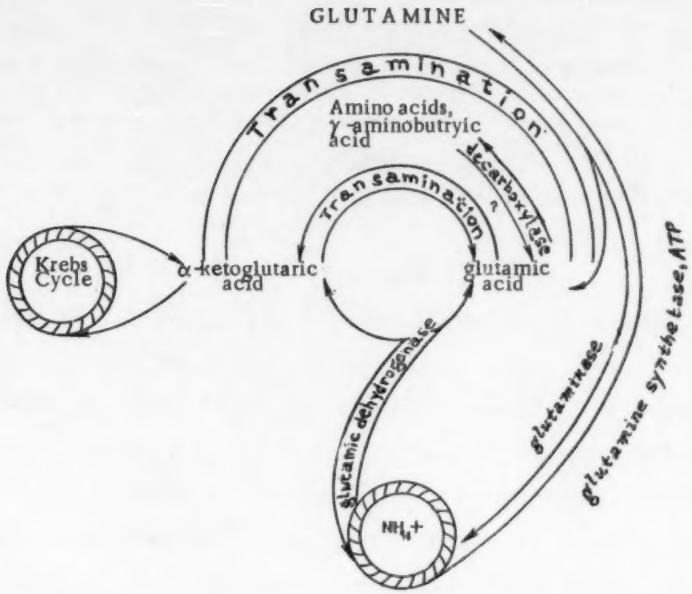
Amino acids	30 min. exposure					2 hr. exposure				
	NH_4-KG	K-KG	H_2O	Difference in μM compared with the water control		NH_4-KG	K-KG	H_2O	Difference in μM compared with the water control	
				NH_4-KG	K-KG				NH_4-KG	K-KG
Expt. 1. Vacuum infiltration of the upper portion of the seedlings										
Glutamic acid	3.11	3.79	2.1	+6.6	+11.0	3.5	5.82	2.6	+5.6	+21.4
Glutamine	1.42	0.45	1.4	0	−6.6	7.23	2.72	3.56	+24.4	−5.7
Aspartic acid	0.73	0.50	0.76	0	−1.8	1.20	1.72	1.2	0	+3.1
Alanine	2.62	2.10	1.8	+9.2	+3.3	3.00	3.00	3.09	0	0
γ-Amino butyric acid	1.72	2.44	1.42	+2.9	+9.9	2.06	2.53	1.59	+4.5	+9.1
Serine	0.79	0.79	1.08	−2.7	−2.7	3.13	3.17	4.17	−9.9	−9.9
Extp. 2 Transport into the upper portion of the seedlings										
Glutamic acid	3.82	4.38	1.91	+13.0	+16.0	2.11	3.83	1.54	+3.87	+15.6
Glutamine	5.32	2.47	3.02	+15.7	−3.77	5.25	3.18	2.62	+18.0	+3.84
Aspartic acid	0.79	1.27	0.84	−0.38	+3.26	1.19	1.50	0.90	+2.2	−4.54
Serine	1.35	1.12	1.08	+2.57	+0.38	2.90	2.16	2.15	+7.14	0.0
Alanine	1.5	1.27	1.27	+2.58	0	0.84	0.84	1.03	−2.14	−2.14
γ-Amino butryic acid	1.42	1.72	1.50	0	+2.1	1.68	1.59	1.31	+3.6	+2.7

content was especially large with introduction of potassium α-ketoglutarate. As in pea seedlings, glutamine was utilized in the process of transamination with α-ketoglutarate. With introduction of ammonium α-ketoglutarate a decrease in glutamic acid content was observed. This decrease is once again the result of the utilization of glutamic acid formed from α-ketoglutarate in glutamine synthesis.

In view of the fact that a perceptible amount of γ-aminobutyric acid is present in the wheat plant [8], we determined this compound in our material. It was established that glutamic acid synthesis occurring as a result of introduction of α-ketoglutaric acid into the tissues is accompanied by the formation of γ-aminobutyric acid. Thus, an increase in the tissues of the glutamic acid concentration leads to an increase in its decarboxylation, which is accompanied by a certain accumulation of γ-aminobutyric acid and is catalyzed by an active glutamic decarboxylase found in cereal grasses [9].

The data obtained enable us to form certain conclusions having an over-all significance for the physiology and biochemistry of nitrogen metabolism in plants. First, it should be emphasized that in the process of assimilation of ammonia and its incorporation into various organic nitrogen compounds, a vital role is played not only by glutamic acid, synthesized from α-ketoglutarate and ammonia in the presence of glutamic dehydrogenase, but also by glutamine, which is synthesized extremely rapidly from glutamic acid and ammonia in the presence of glutamine synthetase. This is also clearly shown by data obtained in our laboratory in the course of an investigation of the incorporation of nitrogen-labeled ammonia by plants [10], and by data from the experiments of Kretovich and Uspenskaya [7], who demonstrated that if ammonium chloride is introduced into a ripening wheat head there is a rapid glutamine synthesis.

Our results also indicate that in living plants glutamine is the main source of amino groups for transamination to keto acids. Here it should be pointed out that utilization of glutamine in transamination is also found upon introduction into a maturing head of phenylpyruvic acid [7]. What is the mechanism of transamination



Scheme of synthesis and conversions of glutamic acid and glutamine in plants.

between glutamine and keto acids in living plant tissues? Is it involved in coupled transaminations [11, 12] or in some other way -- further investigations should indicate thos. In any case, it is clear at present that the scheme of conversions of glutamic acid and glutamine in plants proposed by Chibnall [13] and Pryanishnikov [14] should be revised and extended in the sense that to glutamine should be assigned the corresponding role as the most important source of amino groups, which are utlized in the synthesis by transamination of a variety of amino acids and other nitrogen compounds.

The results of this study and also the data obtained in a study of amino acid synthesis from phenylpyruvate in maturing wheat heads [7] beautifully illustrate the view that in living tissues metabolites simultaneously undergo various enzymatic conversions. Thus, α-ketoglutaric acid introduced into plant tissue undergoes conversions in the Krebs cycle, is aminated by ammonia in the presence of glutamic dehydrogenase, and enters into transamination reactions with amino acids and amides, primarily glutamine. Glutamic acid undergoes amidation with ammonia, decarboxylation with the formation of γ -aminobutyric acid, and transamination. Glutamine is utilized in transamination reactions with keto acids and may at the same time be subjected to deamidation by glutaminase.

The amount of a given metabolite in a plant depends, thus, on the relative rates of enzymatic reactions in which it is involved and on nutritional conditions.

On the basis of our data and published material, the following scheme of synthesis and conversions of glutamic acid an glutamine in plants is proposed (see Figure).

It is understood that the possible interactions of glutamic acid and glutamine in the course of thier transaminations with keto acids are not included.

SUMMARY

Transformations of amino-acids in green pea and wheat seedlings into which solutions of ammonium or potassium — α-ketoglutarate were introduced were studied by quantitative paper chromatography.

Introduction into the seedling of potassium α-ketoglutarate results first of all in the disappearance of glutamine; which takes part in transamination with α-ketoglutarate. The introduction of ammonium α-ketoglutarate results not only in synthesis of glutamic acid but also in very intense synthesis of glutamine.

In wheat seedlings the synthesis of glutamic acid from α-ketoglutarate is accompanied by a certain increase in γ-aminobutyric acid content due to the action of glutamic acid decarboxylase.

The results of this work and of other investigations which were carried out in our laboratory show that in living plant tissues glutamine is not only the most important organic compound in which ammonium ions are fixed, but also a very important source of amino groups for transaminations with keto acids.

The Chibnall-Pryanishnikov scheme of biosynthesis and transformations of glutamic acid and glutamine must be completed by taking into consideration the great importance of glutamine in transamination reactions with keto-acids.

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THE EFFECT OF PRESOWING DROUGHT HARDENING OF WHEAT CARYOPSES ON INDUCED DORMANCY AND GERMINATION

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The effect of repeated soaking and drying of seeds has been known for a long time. This phenomenon has been studied by Saussure [7], Will [8], Genkel' and Kolotova [3] and others, and they have been responsible to a large extent for its explanation.

The purpose of this study was twofold — to explain the ability of imbibed seeds, as well as seeds in the first stages of embryo growth, to enter a dormant state as a result of drying, and to explain the effect of drying of seeds on certain plant properties.

Caryopses of Mercury wheat served as experimental material. First they were carefully selected so that the weight of each caryopsis was 50 mg. Coloring of all the seeds was identical. The selected seeds were divided into six equal groups each of which received a different experimental treatment. The first group was left as a control and untreated; the second was soaked for six hours; the third was soaked to the point of incipient germination; the fourth — to appearance of the central embryonic rootlet; the fifth — to appearance of three embryonic roots,and the sixth — to the piercing of the coleoptile and the emergence of the first true leaf. After the seeds had been soaked a certain length of time or had reached a given stage of embryo growth, they were transferred to a dry ventilated place at a temperature of 20°, where they were dried to the air-dry condition. Seeds thus treated were used in experiments.

It is natural that the question of how long a period during germination the caryopses retain the capacity to resume the dormant condition should arise. In this connection the studies of Erhart [5] and Will [8], who correlated this capacity with the length of the rootlets and the plumule, give us certain hints. In our study we correlated the capacity of the caryopses to enter into secondary dormancy with developmental stages. As a measure of this capacity we used seed viability. In order to determine viability, seeds were taken 1, 10, 20 and 30 days after drying. They were placed in Petri dishes, with four lots of 100 seeds for each experimental group. The samples were kept in a thermostat at 25°. Observations and waterings were performed each day at the same time. The experiment was prolonged for six days in every case. The results obtained are presented in Table 1.

The data in Table 1 show that secondary dormancy can be induced in wheat caryopses after seed swelling and even after germination. The seeds are unable to resume the dormant condition after formation of the coleoptile and emergence of the first true leaf.

Our results confirm the fact established by Makek[6] that shoots retain their viability for a longer time than roots. Ordinarily those parts of the roots which have already emerged from the coleorhiza die.

Of the shoot organs, the coleoptile first loses its viability. The first indications of a disturbance in its development were observed in shoots from caryopses which had been dried after appearance of the three embryonic roots. In certain cases it was observed that after a second soaking the coleoptile, though still alive, did not grow further. This was sometimes associated with the death of the upper cells of the coleoptile. The next step in this direction was complete loss of viability by the coleoptile.

The death of the roots did not entail the death of the plant as a whole. On the contrary, new roots appeared shortly before the second soaking.

TABLE 1

Viability of Caryopses of Mercury Wheat after Drying at Various Stages of Germination

Treatment	Number of days after drying			
	1	10	20	30
Control	98	98	98	98
Soaked 6 hr	95	95	95	98
Incipient germination	96	90.5	94	97
With 1 root	93	90	95	94
With 3 roots	93	97	90	92
With 1 true leaf	0	0	0	0

TABLE 2

Percent of Wheat Caryopses Germinating in Soil After Drying at Various Growth Stages

Treatment	Number of days after sowing	
	6	30
Control	0	90
Soaked 6 hr	0	94
Incipient germination	4.7	95
With 1 root	7.1	90
With 3 roots	8.7	88

TABLE 3

Indices of Wheat Growth Measured the 30th Day After Sowing of Seeds Which Had Been Dried at Various Germination Stages

Treatment	Stem length, in cm	Maximal root length, in cm	Dry wt, in mg
Control	6.6	15	22.2
Soaked 6 hr	6.6	15	20.5
Incipient germination	6.25	17.5	21.2
With 1 root	6.5	16.7	27.7
With 3 roots	6.7	11.8	24.2

TABLE 4

Amylase Activity and Absorptive Capacity of Caryopses After Drying at Various Stages of Germination

Treatment	β-amylase activity (maltose formed, mg)	Absorptive capacity (wt of water absorbed, g*)
Control	17.2	0.140
Soaked 6 hr	19.0	0.170
Incipeint germination	25.1	0.250
With 1 root	27.3	0.290
With 3 roots	28.6	0.370

* Determined in 2 molar NaCl.

We also studied the growth of caryopses in soil in connection with their ability to enter a second dormant period. Seeds treated in various ways were sown ten days after being dried. For this we used pots containing 8 kg of soil. In each pot 50 seeds were planted. The individual treatments were replicated eight times. A count of germinating seeds was made on the 6th and the 30th day after sowing. The results obtained are presented in Table 2.

As Table 2 shows, not only soaked seeds but also germinated seeds are able to grow vigorously after drying. On the sixth day, the seeds which had one or three roots before drying had shown the greatest growth. This picture is, however, markedly different on the 30th day. The highest percentage of germinating seeds is found in the group which had been dried at the stage of incipient germination. It is interesting to note that in the group whose seeds had been dried after the appearance of three roots, the percentage of germinating seeds was somewhat lower than that of the controls.

On the 30th day the stem length, the maximal root length and the dry weight of the plant were measured. The results obtained are presented in Table 3.

As Table 3 shows, the treated plants and the controls have almost identical stem lengths. Differences between treatments are greater with respect to root length and dry weight. Seeds which were dried at the time of incipient germination show the greatest deviation from the controls. After them come those which were dried at the single root stage. The remaining treatments are almost indistingushable from the controls.

These results would not be intelligible if changes which had occurred during swelling and germination prior to drying were not taken into consideration. For example, the more rapid germination of seeds which had been dried at later stages of embryo growth may be explained to a certain extent by the fact that the shoots remain completely viable. The death of protruding roots does not entail the death of the seedling as a whole because the young plant is nourished primarily by its nutrient reserves and also because under favorable conditions new embryonic roots, and later adventitious roots, are easily formed.

Significant changes in amylase activity and absorptive capacity occur in the seeds (Table 4).

For the determination of β-amylase, 2% extracts of meal obtained from experimental and control seeds were prepared. Dissolved starch was hydrolyzed by these extracts in the presence of acetate buffer (pH 5). Reducing substances were determined by the ferricyanide method (using maltose as a standard).

As Table 4 shows, the greater the embryo growth before drying, the more marked the increase in amylase activity and absorptive capacity of the seeds. Those seeds which were dried at later stages of development absorbed the necessary quantity of water from the soil more rapidly and the biochemical processes underlying germination were thereby promoted. It is undoubtedly true that the increased level of amylase activity also plays a significant role in the hastening of seed germination.

While these facts help us to understand why seedlings dried at advanced stages of growth grow more rapidly, they do not explain why such seedlings are capable of entering a state of secondary dormancy. In answering this question we used as a point of departure the assertion of Genkel' [1] that one of the preconditions of transition to the dormant state is the accumulation of the necessary amount of nutritional reserves. Using the experimental procedures of Genkel' and Oknina [4], we studied changes in sugars and fats in cells of the embryo. Sections were cut on a freezing microtome, treated with the proper reagents and studied under the microscope.

Our observations established that sugars occur in very minute amounts in cells of the embryo irrespective of seed treatment. Changes in the fat complement are of greater interest. If they are followed throughout the germination period, it can be seen that as growth of the embryo proceeds the dense layer of fats becomes progressively more broken up into droplets; this occurs with all treatments. This process occurs at different rates in various parts of the embryo. It is most rapid in the roots. Those roots which have emerged from the coleorhiza are almost completely free of accumulated fats. Only traces are present, and in some cases fats are totally absent. Next in order with respect to rate of fat utilization is the coleoptile; in this case, however, the rate is considerably lower than in the roots. Fats are utilized most slowly in the intermediate portions of the seedling in the region of the plumule and hypocotyl.

TABLE 5

Percent of Dissolved Material in the Cell Sap and Permeability of the Protoplasm of Aerial Portions of Plants Given Different Experimental Treatments

Treatment	Percent of dissolved material in the cell sap	Resistance, in ohms (× 1000)	Treatment	Percent of dissolved material in the cell sap	Resistance, in ohms (× 1000)
Control	13	39	Incipient germination	16	60
Soaked, 6 hr	14	55	With 1 root	14	55
			With 3 roots	12	40

These findings indicate that the capacity of seeds to become secondarily dormant following swelling and even slight germination bears an extremely close relation to the presence of reserve fats in the cells. Those cells which have utilized their fats die with drying since they have already emerged from dormancy and have apparently established protoplasmic connections, plasmodesmata, among themselves.

We were also interested to know what effect seed drying had on the frost resistance of plants grown from such seeds. On the tenth day after drying, seeds were sown in flats. Each flat contained 7 kg of soil. For each seed treatment, six lots of 50 seeds each were sown. When the young plants had reached the third leaf stage, they were transferred to a room at a temperature of −16° and left there 24 hours. During the succeeding 48 hours, the temperature was gradually increased, and the flats were then transferred to a greenhouse at 10-15°. One month later the flats were then transferred to a greenhouse at 10-15°. One month later the living plants were counted. The following results were obtained:

Experimental treatment	Percent of living plants
Control	2.04
Soaked 6 hours	12.75
Incipient germination	25.20
With 1 root	15.20
With 3 roots	00.00

These data indicate that drying of seeds which have been soaked as well as germinated seeds may result in greater frost resistance in certain cases. An especially beneficial effect on seeds dried at the point of incipient germination was noted, and this corresponds with the findings of Genkel' and Kolotova [2] with respect to resistance of hardened plants to freezing.

All the plants which had undergone drying at the stage of emergence of three embryonic roots succumbed to freezing. Plants in the remaining experimental groups exhibited a somewhat higher frost resistance than control plants.

On the day that the flats were transferred to a cold room, samples from the aerial portions of the plants were taken. The cellular sap was expressed from the samples and the amount of dissolved material determined with a refractometer. In addition the permeability of the protoplasm was measured with a Wheatstone bridge. The results obtained are summarized in Table 5.

As Table 5 shows, the various experimental treatments fall into almost the same order with respect to percentage of dissolved material in the sap and protoplasmic permeability as is the case with frost resistance.

SUMMARY

Wheat caryposes are capable of entering the dormant condition after soaking and even slight germination. This property is retained until the coleoptile is pierced and the first true leaf appears.

An especially important factor in the transition of embryo cells of wheat caryopses to a state of secondary dormancy is the presence of storage fats. Cells not containing fats have apparently already established intercellular protoplasmic connections; they are unable to enter the dormant condition and they therefore die. Root cells lose their fats most rapidly, and upon drying they are the first to die.

The properties of plants grown from seeds which were dried after soaking or even after germination depend to a large extent on the stage at which the drying was carried out. Drying exerts an especially favorable effect on caryopses which are at the stage of incipient germination. Plants grown from such caryopses develop most vigorously, accumulate the greatest amount of dry matter, and are most frost resistant.

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FORMATION OF FLOWER BUDS AND DIFFERENTIATION OF FLORAL PARTS IN APPLE

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The formation of flower buds has been studied by many workers. The first observations were made in Russia by Zheleznov [1] in 1851. He showed that flower buds are formed in the year preceding flowering; during the winter there is a marked increase in dry weight of the buds and in the dimensions of floral parts as well as a change in their form; in addition certain new structures are formed. Development of flower buds during the winter has also been observed by other investigators [2, 3]. Kobel' [4] gives a detailed review of work on this subject performed since 1877.

In the Soviet Union the most complete studies of flower bud formation and development of floral parts have been made by Ro [5].

Since 1949 we have carried on investigations to determine the times at which flower buds are laid down and floral parts differentiated in 23 varieties of apple in the Krasnodar region.

Dates on which flower buds were laid down were determined by studying their structure microscopically and by a graft method. Buds for microscopic study were collected at the following times: every 5-8 days from June 1 to September 25, every 10-15 days from September 26 to November 15, and once a month during the winter. At each collection, 10 buds of each variety were taken. Buds were sectioned longitudinally and sections were examined under the microscope at a 50-fold magnification.

The first sign that a given bud will be floral is the enlargement of the meristematic area. Terminal and lateral flower primordia are then formed. The fourth stage is the formation of the sepals, the fifth, of the petals, the sixth, of the anthers, and the seventh, of a rudimentary pistil from the fertile leaves.

The average dates of formation of floral buds and differentiation of floral parts are given in Table 1.

The generally used method of determining times of floral bud formation and differentiation of floral parts by studying their structure has great disadvantages, the chief of which is the destruction of the object studied (bud). In addition to this method, we used the method of grafting spurs onto 10-20-year-old stocks which had not become dormant and which had great growth potentialities.

Spurs from buds laid down in the current year were grafted every 15 days from June until late autumn. Under these conditions, the scions had been stimulated to grow, and the floral buds had produced inflorescences and the leaf buds shoots, 20-30 days after grafting.

It is well known that floral buds laid down in one year ordinarily do not grow out until the succeeding year. With respect to this phenomenon, Sergeev [2] asserts that during the dormant period floral and leaf buds pass through a developmental stage for which lowered temperatures are necessary. He claims that without exposure to these temperatures floral buds do not develop but degenerate and dry up. The known instances of premature flowering he explains in terms of a physiological aging of the buds.

Our investigations [6] have shown that with an abundant supply of nutrients the floral buds are rapidly induced to grow and begin flowering without exposure to cold. Spurs grafted in the usual way are stimulated

TABLE 1

Times of Formation of Floral Buds and Differentiation of Floral Parts in Apple (in days)

Variety	Enlargement of meristem (1)	Formation of terminal flower primordium (2)	Formation of lateral flower primordia (3)	Formation of sepals (4)	Formation of petals (5)	Appearance of anthers (6)	Growth of fertile leaves (7)
Michurin seedless	24.VI	5.VII	11.VII	1.VIII	9.VIII	30.VIII	12.IX
Borovinka	28.VI	5.VII	11.VII	27.VII	10.VIII	30.VIII	5.IX
Bel'fler-china	28.VI	12.VII	17.VII	31.VII	16.VIII	30.VIII	15.IX
Irish peach	24.VI	10.VII	18.VII	29.VII	13.VIII	27.VIII	5.IX
Kandil'-china	2.VII	12.VII	17.VII	31.VII	23.VIII	3.IX	12.IX
Melba	28.VI	10.VII	16.VII	1.VIII	21.VIII	31.VIII	8.IX
Saffron pippin	2.VII	11.VII	26.VII	13.VIII	20.VIII	2.IX	17.IX
Wealthy	5.VII	16.VII	27.VII	9.VIII	23.VIII	2.IX	17.IX
Snowy	28.VI	10.VII	22.VII	——	12.VIII	20.VIII	8.IX
Sedley	2.VII	13.VII	22.VII	6.VIII	27.VIII	7.IX	17.IX
Golden winter parmen	8.VII	22.VII	28.VII	13.VIII	——	1.IX	24.IX
Ben Davis	8.VII	25.VII	10.VIII	21.VIII	27.VIII	5.IX	17.IX
Lavfam	11.VII	20.VII	27.VII	20.VIII	30.VIII	9.IX	20.IX
Leafy pippin	8.VII	14.VII	22.VII	9.VIII	27.VIII	3.IX	15.IX
Winesap	11.VII	26.VII	21.VIII	9.IX	—	25.IX	10.X
Mac Intosh	8.VII	16.VII	3.VIII	27.VIII	3.IX	12.IX	27.IX
Golden transparent	14.VII	26.VII	14.VIII	24.VIII	4.IX	5.IX	20.IX
Aesop Spitzenburg	16.VII	21.VII	14.VIII	21.VIII	28.VIII	4.IX	15.IX
Trebu seedling	16.VII	21.VII	1.VIII	14.VIII	28.VIII	4.IX	25.IX
Transparent	14.VII	25.VII	13.VIII	2.IX	——	22.IX	1.X
Jonathan	16.VII	24.VII	20.VIII	5.IX	7.IX	22.IX	1.X
Winter banana	16.VII	27.VII	10.VIII	24.VIII	5.IX	20.IX	5.X
Boiken	14.VII	29.VII	27.VIII	4.IX	9.IX	20.IX	21.X

Note: Figures in parentheses following the column headings denote stages of development of floral buds.

to grow if the graft is made in early spring, when the stock is active. Spurs grafted in the summer being to grow, as a rule, only in early spring of the following year; this is even more true of spurs grafted in the fall. During the summer-fall period the stock becomes dormant and the flow of nutrients necessary for growth to the grafted spurs or buds is curtailed.

The studies of Dostal' [7] and ourselves have established that the transition of plants to the dormant condition is related to the activity of the leaves. In order, therefore, to maintain the stocks in actively growing condition, we topped 10-20-year-old trees at about a meter from the ground early in the spring at the beginning of the growth period. During the spring and summer we systematically removed shoots from dormant buds in the stump. Such stumps, deprived of leaves, remained active till late fall and supported a rapid growth of the grafted scions during the summer-fall period.

In the study of floral bud formation, about 100 trees on a forest plot occupied with wild apple, which is subject to uprooting, were topped, and more than a thousand spurs were grafted onto the stumps from June to December. For such a graft it was also possible to use discarded apple seedlings on a hybrid plot.

The times of formation of floral buds as determined by study of their anatomical structure were substantially the same as those determined by the graft method. The time of flowering of spurs grafted at various times is shown in Table 2.

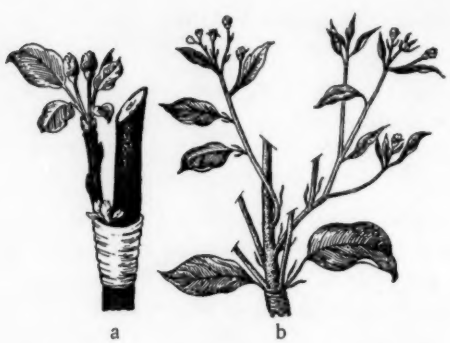
TABLE 2

Formation of Shoots and Inflorescences from Buds Laid Down the Same Year on Spurs Grafted at Various Times

Variety	June 7			June 23			July 23		
	No. of spurs grafted	No. formed from them		No. of spurs grafted	No. formed from them		No. of spurs grafted	No. formed from them	
		of shoots	inflores-cences		of shoots	inflores-cences		of shoots	inflores-cences
Borovinka	20	6	—	20	12	1	18	1	14
White sap	23	9	—	20	11	1	16	2	6
Golden winter parmen	22	6	—	17	10	—	18	1	10
Jonathan	—	—	—	19	13	—	20	5	6

Table 2 shows that spurs grafted on June 7 produced only shoots. At this time floral buds had not yet been laid down. From spurs of Borovinka and White sap which were grafted on June 23 one inflorescence was formed. A study of the anatomical structure of the buds indicated that enlargement of the meristem had occurred, but the floral primordia had not as yet appeared. In these varieties an extensive formation of inflorescences was observed in spurs grafted from July 7 on. Spurs of varieties Golden winter parmen and Jonathan flowered only with grafts made on July 23 or later. Grafts of Bel'fler-china and Snowy made on July 23 also formed inflorescences – 15 out of 17 in the first case and 14 out of 16 in the second case. As a rule grafted spurs of all varieties produced inflorescences at later dates. After formation of floral primordia grafted buds develop as floral buds even in the absence of leaves.

The times of differentiation of various floral parts in grafted spurs and nongrafted spurs are different. While ungrafted floral buds complete the formation of all the principal floral organs in September or November and flower only in the spring of the following year, in grafted buds all floral parts are formed and developed in the course of 30-40 days.



a b

Fig. 1. Dependence of structure of the inflorescence of White sap apple on the stock. a) Cutting grafted on a two-year-old stock in the dormant condition; b) cutting grafted on a 20-year-old stock in an actively growing condition.

The times at which formation and differentiation of floral parts take place depend on the flow of nutrients necessary for growth to the developing flowers. With an abundant supply of nutrients, the buds are able to form floral parts rapidly without exposure to cold.

Under natural conditions, premature flowering seldom occurs, since at the time of floral bud formation the whole plant begins to enter a dormant state, in which metabolism and translocation are depressed. Premature flowering occurs in nature, then, when the transition of the plant to a dormant condition is prevented, or under the influence of external conditions (rain after a drought, removal of a branch above a bud, etc.), which bring an increase of flow of nutrient substances to the buds.

As already pointed out the activity of the leaves at a definite stage induces dormancy. In cases of premature leaf fall after the onset of floral bud formation (end of July – August), therefore, the transition to a dormant condition is delayed. Such plants may renew their growth and flower.

Fig. 2. Change in apple flowers under the influence of a vigorous stock.
a) Conversion of sepals into leaves; b) stamens into petals; c.) and d) petals into leaves; e) flowers into shoots.

Fig. 3. Formation of underdeveloped fruits on fertile excrescences from vegetative parts.

The structure of an inflorescence of a single variety of apple depends on the availability of nutrient substances during setting of floral buds and flowering. Apple forms solitary flowers, corymbose inflorescences, or branched complex inflorescences with strongly developed vegetative parts.

In 1956, certain Melba apple trees suffered severely from leaf mottling, and in July the leaves fell; in August growth resumed and the trees flowered prematurely. Because of a severe drought occurring at this time growth was very feeble, and inflorescences were underdeveloped with a small number of flowers. Of 656 inflorescences, 135, 20.6%, were one-flowered; 187, 28.5%, were two-flowered; 199, 30.3%, were three-flowered; 82, 12.5%, were four-flowered, and 53, 8.1%, were five-flowered. These inflorescences had on the average 2.6 flowers; under normal conditions one- and two-flowered inflorescences do not occur in this variety, and the average number of flowers in an inflorescence is 5.5.

The number of flowers in an inflorescence also depends on the stock. Spurs grafted onto dormant stocks form in general one- and two-flowered inflorescences (Fig. 1a). On such stocks the flow of nutrient materials necessary for growth does not occur, and therefore the rudimentary lateral flowers often do not develop but dry up. Such spurs grafted onto vigorous 10-20-year-old stocks, however, form six- to seven-flowered inflorescences.

The number of flowers in an inflorescence is directly related to their productivity. Our findings indicate that one- to three-flowered inflorescences often fail to form fruits, and four-flowered inflorescences form them to only a small extent (up to 3.8%). Seven- and eight-flowered inflorescences are the most productive. Fruit production on these inflorescences is as high as 28.6%. In the cultivation of fruit trees, therefore, the means of encouraging formation of the most productive inflorescences should be considered.

Spurs grafted on a vigorous stock often form branched inflorescences with elongated axes, attaining a length of 10 cm and more, and with a large number of growing shoots. The character of flowering of White sap on a vigorous, actively growing stock, is shown in Fig. 1b.

In flowers which have been formed on such stocks, considerable alterations of form, consisting of a transition from the normal flower to a vegetative shoot, are observed. Such alterations are manifested in an elongation of the pedicel, a conversion of sepals and petals into leaves and of stamens into petals, and, in the extreme case, of a flower into a shoot. These changes are illustrated in Fig. 2. They are the result of a reverse development of reproductive organs into vegetative organs. Under natural conditions, such changes are also induced by an increased flow of nutrients to the flowers.

Sergeev [8], referring to published information and his own observations, asserts that lowered temperatures of the winter period are necessary for the formation of archesporial tissue. This conclusion is not supported by our work. Pollen grains obtained on August 28 from prematurely flowering Melba apple trees germinated in a 15% sugar solution and produced tubes of normal length. The germinability of such pollen was lower, however, than that obtained from trees flowering at the normal time.

Cases of formation of fruits with seeds from premature flowers indicate that archesporial tissue may be formed in the absence of exposure of buds to low temperatures.

Our studies have established that the formation of underdeveloped seedless fruits on fertile patches from vegetative parts can occur (Fig. 3). We have observed this in the Labin state fruit and berry farm of the Krasnodar region, in the state orchard of the Sclavonian district and in the nursery of the Maikop Experimental Station of the All-Union Institute of Plant Cultivation. These cases are seldom encountered, however, and have no practical significance.

SUMMARY

The dates of setting of flower buds and of differentiation of floral parts in 23 varieties of apple trees have been studied at the Maikop Experimental Station of the All-Union Institute of Plant Cultivation since 1949.

A study of the anatomical structure of buds collected at various periods indicated that in the foothills of the Krasnodar pegion flower buds begin to set at the end of June or in July.

The first indication that a given bud is beginning to develop as a flower is expansion of the apical meristem. In various varieties of apple trees this phase was found to occur between June 24 and July 16. In September – October all of the main parts of a flower are present in the flower bud.

The date of setting of the flower buds was determined not only by anatomical means but also by grafting spurs on powerful stocks which had not entered the dormant state. The active state of 10-20 years stocks during the summer and until late fall can be maintained by removing the crown of the stock in early spring since absence of foliage retards the plants going over to the dormant state.

In flower buds grafted onto powerful stocks which ensure an inflow of nutrients, differentiation and development of all parts of the flower is compleated within 30-40 days, whereas under natural conditions this period lasts 9-10 months.

In grafted cuttings with floral primordia further differentiation of floral parts can proceed without the leaves.

With an abundant supply of nutrients to the flower bud, all parts of the flower can develop without the application of low temperature encountered in the winter. The number of flowers in an inflorescence and also the floral structure depends not only on varietal peculiarities but also on the flow of nutrients to the bud. If this flow is poor, 1 or 2 inflorescences with underdeveloped flowers are formed, whereas when the flow is abundunt multiflorous and frequently complex inflorescences are formed with enlarged axes, pedicels, sepals and other floral parts. Abundant flow of nutrients to the flower buds may also lead to the conversion of reproductive organs into vegetative ones, even to complete transformation of the flowers into shoots.

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EFFECT OF SOIL MOISTURE DEFICIENCY ON GRAIN RIPENING IN SPRING WHEAT

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In wheat, the period from flowering to the waxy stage, which is the period of concentration in the grain of nutrient material accumulated by the plant and of development of assimilative capacity in the grain, is exceptionally important with respect to yield. At this time, weather conditions are often unfavorable, and this leads to a distrubance of the normal course of formation and development of grain with resulting grain damage.

Factors causing grain damage have been adequately described in published papers [1-3]. Drought causes the greatest damage to normal grain ripening. Although the effect of drought on plants has been studied by many workers, the period of grain development in wheat has a number of special features which oblige one to speak of causes of yield reduction individually. At the time of grain formation and ripening, the increase in weight of vegetative tissue ceases and the translocation of nutrient material from the vegetative portion of the plant to the grain assumes great importance.

The purpose of this study was to evaluate the effect of soil moisture deficiency occurring at the time of grain development on the weight of the vegetative portion of the plant, on grain maturation, and on water content of the vegetative mass and of the grain in order to arrive at an estimate of the principal causes of yield reduction. The role which photosynthesis plays in the production of dry matter in the grain during grain ripening was also studied. It was assumed that even varietal differences may be of value in the assessment of drought resistance of varieties during grain development.

METHODS

The study is based on growth experiments performed in 1954 and 1955 at the P.I. Lisitsyn Breeding and Genetics Station in the Timiryazev Agricultural Academy. Experiments were performed using Wager flats. Each flat contained 5.25 (1954) or 5.00 (1955) kg of absolutely dry soil. The soil used was a light, coarse-sandy clay loam. Ten plants were grown in each flat. Soil moisture was maintained at 65% of the saturation level. Just before formation of the grain was complete, watering of the experimental plants was stopped in order that at the end of the formation period and the beginning of the milk stage the soil moisture would be at a level equal to twice the maximal hygroscopicity. At this time permanent wilting of the plants occurred. They were left in this condition for five days after which normal watering was resumed. The experiments were replicated three or four times.

Total nitrogen was determined by the Kjeldahl method. A difference between parallel determinations of more than 0.1% nitrogen rendered the results invalid.

The essential feature of our growth experiments was the collection of plant samples during ripening at the time of resumption of irrigation of the experimental plants (three flats – occasionally two – were taken from each experimental group). In this way it was possible to follow the effect of drought on the accumulation of dry matter in the grain and on other processes, and not merely to rely on final results. In growth experiments, which afford a more precise comparison with controls than do field experiments, this is so much to be desired that it is necessary to complicate the experiment by the addition of extra flats.

Samples were fixed with steam and subsequently dried at 50-60°. For determination of moisture content, a single grain, the first or second, was taken from the middle of each head.

Our conclusions are based either on statistical proof or on the repeatability of the results, even though these were not statistically evaluated in each individual case. Figures in the tables refer to the aerial portions of the main stem.

RESULTS

In the growth experiments (Table 1), drought induced a marked loss of water by the plants. It should be noted that the maturing grain is less subject to water loss than is the vegetative mass. This is evidently related to the greater physiological activity of the grain at this time as compared with other parts of the plant. It is to be expected, therefore, that it would suffer less from drought in other ways as well. Actually, loss of water by the vegetative organs led to a marked decrease in their weight. This can be attributed, primarily, to the enhancement of catabolic processes brought about by water depletion and to a heightened utilization of the resulting products in respiration [4, 5]. At the same time, a portion of these products was utilized in grain maturation. This serves to explain the fact, at first glance paradoxical but undoubtedly correct, that the rate of grain maturation increases, or at least remains unchanged, during short periods of drought (increase in comparison with the control of the weight of 1000 grains in the experimental group after the drought period). The same results were obtained in 1954 by subjection of both varieties to a five-day dry period at the end of the milk stage (not presented here in the interests of brevity).

TABLE 1

Data from Growth Experiments with Spring Wheat, Varieties Moscow and Lyutestsens 62

Variety and year of expt.	Date of determinations*	Moisture content of stem tissue (of plants in 1954) in %		Moisture content of grain in %		Wt of vegetative tissue of 10 stems in g		Wt of 1000 grains in g	
		control	drought	control	drought	control	drought	control	drought
1954									
Moscow	I	61.8	58.2	64.5	63.0	15.4	12.6	16.3	16.0
	II	—	—	—	—	10.9	10.7	38.1	36.4
Lyutestsens 62	I	64.0	62.4	64.1	63.7	16.2	14.9	13.7	15.2
	II	—	—	—	—	11.5	10.9	36.0	35.1
1955									
Moscow	I	60.8	48.9	64.7	60.2	19.6	16.5	14.8	14.8
	II	—	—	—	—	15.9	15.8	36.6	30.7
Lyutestsens 62	I	63.8	53.8	60.0	62.8	19.5	16.5	10.8	11.5
	Ia	61.5	56.0	62.8	64.7	17.7	16.4	14.8	14.8
	II	—	—	—	—	13.5	12.1	33.0	30.3

* I) After the drought period; Ia) three days after the drought period; II) at full maturity.

A marked rise in the dry weight increment of grain resulting from a short dry period was also noted in the course of observations of grain ripening in the field in 1952 and 1953. A collection of 100-200 heads was made daily from the milk stage to the stage of full maturity; the heads were fixed with steam and dried, and after two months the weight of 1000 air-dry grains was determined.

In 1953, soon after the beginning of the milk stage, a soil moisture deficiency was noted. Leaves active at this time began to yellow and wither. The soil moisture level from 0-40 cm was about double the maximal hygroscopicity. Plants remained in this condition five days until a heavy rain on July 16.

The period of movement of nutrient materials into the grain was thus divided by the July 16 rainfall into two parts: in the first, maturation proceeded under conditions of decreasing water availability, and in the second it proceeded under conditions of adequate water supply. If the corresponding period in 1952, a wet year, is divided into parts of the same length, then the increase in the rate of ripening following a period of water deficiency becomes quite striking (Table 2).

TABLE 2

Field Data on Grain Ripening in Spring Wheat, Varieties Moscow and Lyutestsens 62

Year	Period of grain development	Duration in days	Wt. of 1000 grains at the end of period	Moisture conditions prevailing
		Moscow		
1952	3.VII — 23.VII	20	16.2	Sufficient moisture
1953	26.VI — 16.VII	20	19.4	Drought
1952	23.VII — 8.VIII	16	42.3	Sufficient moisture
1953	16.VII — 1.VIII	16	37.0	Sufficient moisture
		Lyutestsens 62		
1952	30.VI—23.VII	23	18.6	Sufficient moisture
1953	23.VI—16.VII	23	23.8	Drought
1952	23.VII— 6.VIII	14	36.9	Sufficient moisture
1953	16.VII—29.VII	13	33.9	Sufficient moisture

The difference in the average daily air temperature in the two years did not exceed 0.9°, and could not substantially affect the rate of movement of nutrient materials into the grain.

The increase in the rate of ripening which occurs during a drought period is based on a premature, rapid degradation of vegetative tissues, according to data obtained in growth experiments.

Mothes [6] has shown that during wilting proteolytic processes in the lower leaves are enhanced and soluble nitrogen compounds are translocated to young leaves. In addition, Trubetskova and Semenova [7, 8], who generalized the results of their own experiments on wheat and barley and those of other workers, came to the conclusion that during a drought period there is a transfer of nitrogen from older to younger organs. Depending on the stage of plant development at the time of drought, there was either an increase in the tillering coefficient or in the percent of nitrogen in the grain. The latter was noted when drought occurred during the shooting period [8], and even when it occurred during the tillering stage [9]. This is even more to be expected if drought occurs during the grain ripening period, at which time the mobile nitrogen compounds produced by proteolysis may be immediately assimilated by the grain.

The change in nitrogen content of grain resulting from drought which was noted in our growth experiments is shown in Table 3.

TABLE 3

Total Nitrogen Content of Grain in the Growth Experiments of 1954 and 1955

Variety and year	Time of measurement	Total nitrogen			
		in %		in 1000 grains, in g	
		control	drought	control	drought
1954					
Moscow	After drought	1.90	2.14	0.31	0.34
	At full maturity	1.95	1.96	0.74	0.71
Lyutestsens 62	After drought	2.21	2.33	0.30	0.35
	At full maturity	2.22	2.30	0.80	0.81
1955					
Moscow	After drought	1.76	2.40	0.26	0.36
	At full maturity	1.68	2.02	0.61	0.62
Lyutestsens 62	After drought	2.06	2.49	0.22	0.29
	Three days after the drought period	1.86	2.12	0.28	0.31
	At full maturity	1.77	2.04	0.58	0.62

The percentage content of nitrogen in grain subjected to a five-day drought was considerably higher than that of control grain. The absolute nitrogen level was also higher immediately following resumption of irrigation. This compels one to reject the hypothesis that an increase in percentage content of nitrogen is due solely to a more rapid loss of carbohydrates in respiration during the drought period. It is clear that the amount of nitrogen in the nutrients entering the grain during the drought period increases both relatively, as a consequence of an increased loss of carbohydrates in respiration, and absolutely, as a result of intensified proteolysis in the vegetative organs which releases mobile nitrogen compounds.

Petinov and Pavlov [10], on the basis of a study of the effect of removing leaves from various nodes on the percentage of nitrogen in the grain, are of the opinion that the flow of nitrogen into the grain is less subject to unfavorable moisture conditions than is the flow of carbohydrates. To this may now be added that drought even stimulates the flow of nitrogen into the grain in initial stages. This circumstance should be of some consequence in the increase of grain nitrogen content during drought periods.

TABLE 4

Results of a Growth Experiment of 1955 with Reduction and Complete Exclusion of Light

Wt of vegetative portion of 10 stems, in g			Wt of 1000 grains, in g			Total nitrogen of grain, in %			Total nitrogen of 1000 grains, in g		
control	shaded	light excluded	control	shaded	light excluded	control	shaded	light excluded	control	shaded	light excluded
15.9	14.8	14.9	36.6	34.0	22.3	1.68	1.68	2.62	0.61	0.57	0.58
13.5	14.2	13.2	33.0	31.9	16.6	1.77	1.82	3.30	0.58	0.58	0.55

Note: Upper row of figures refers to Moscow spring wheat, the lower to Lyutestsens 62 wheat.

Whether the increased tempo of grain ripening in drought conditions should be attributed only to an increased flow of nitrogenous compounds into the grain, or whether a more rapid translocation of carbohydrates is also a contributing factor is still unclear. This latter possibility, it seems to us, is not excluded by the experiments of [11] and [12], since they are concerned, apparently, with plants which have been exposed to a long and severe drought.

The absolute amount of nitrogen in mature grain was found to be almost the same in experimental and control plants. A more evenly distributed flow of nitrogen from the vegetative organs promoted a better filling of the grain, however. An excessively rapid outflow of nitrogen means that there is a severe reduction of the active green surface of the plant and this in turn means that the capacity for synthesis of organic material is decreased. That drought exerts an extremely unfavorable effect on photosynthetic activity both immediately and as a result of suppression of growth processes on which the dimensions of the active synthesizing surface depend, has been shown by many studies [4, 5, 11, 13, 14, 15]. With respect to wheat which is in the stage of grain maturation, it is a question, of course, not of suppression of growth processes, but of a severe diminution of previously formed green tissue. However, in order to speak of the suppression of photosynthesis at very late stages of development as a cause of reduced yields, it must be shown that photosynthesis plays a significant role in the manufacture of grain dry matter at these stages.

Investigations of a number of workers [10, 16, 17, 18] show that the activity of various green parts (leaf blades and sheaths, scales of the head) during the period of grain development plays a substantial role in yield production. All these studies were made at a late stage of development and were in general concerned with individual parts of the plant. In Table 4 are presented results of a growth experiment in which some plants were shaded by three layers of gauze stretched on a frame and others were kept under a cardboard box with double walls which was ventilated. Experiments were run in triplicate and water was applied according to weight.

TABLE 5

Results of a Field Experiment with Shaded Plants, 1955

Time of sample collection	Wt of vegetative portion of 100 stems in g		Wt of 1000 grains in g		Total nitrogen content of the grain, in %	
	control	shaded	contr.	shaded	contr.	shaded
Moscow						
Before shading	217.9	222.0	6.8	6.4	2.11	2.22
After shading	227.4	218.9	11.6	10.9	1.81	2.06
At full maturity	177.9	181.0	42.0	40.3	1.78	1.82
Lyutestsens 62						
Before shading	222.5	226.0	4.4	4.4	2.78	2.91
After shading	229.0	212.0	10.1	8.2	2.21	2.72
Three days after shading	220.7	206.4	14.6	12.5	2.02	2.22
At full maturity	166.2	156.2	38.7	35.3	1.94	2.16

Measurements indicated that were no essential differences in temperature and relative humidity among the various experimental set-ups – under the frame, under the box and in the open air. The experiment was begun at a late stage, the beginning of the milk stage.

A reduction in light intensity (2.9-fold as compared to the normal intensity) caused a decrease in the weight of 1000 grains which was, however, insignificant. Evidently, even under reduced illumination photosynthesis is sufficiently rapid. Total exclusion of light resulted in a reduction in grain weight of such an extent as to indicate that a very large proportion of the grain dry matter is formed as a result of photosynthetic activity during the ripening period.

It is known that in the dark there is a vigorous destruction of proteins in old leaves leading to the formation of easily transportable nitrogenous compounds [19, 20]. Obviously this process should be strongly manifested at a stage as late as that of grain maturation. At the same time, it was shown in an experiment in which wheat heads were shielded from light that the translocation of nitrogen into the grain is not impeded [10]. In our experiment the whole plant was shielded from light. Translocation of nitrogen into the grain was essentially complete in the dark: the amount of nitrogen in 1000 grains was approximately the same in experimental and control groups. It is to be expected that the percentage of nitrogen in the grain of the experimental group is very high.

At earlier stages of grain development photosynthesis plays, apparently, a still more prominent role. In 1955 a field experiment was set up in order to study the effect of a short-term reduction of light intensity on the course of accumulation of dry matter in the grain at the stage at which its formation was complete and it had begun to mature. The plants were shielded by three layers of gauze stretched on a frame for four days. Each sample consisted of three groups of 20 main stems. The results are presented in Table 5.

In spite of the differing initial values for control and experimental samples, which are due to the inhomogeneity of the plot, it is clear that a reduction in light intensity brought about an immediate decrease in the rate of maturation. The vegetative portion of the plants was markedly reduced in the experimental group in comparison with the control group. There was an abrupt change in the composition of the materials entering the grain which consisted of an increase in the proportion of nitrogen. As a result, the weight of 1000 grains in the experimental group was markedly lower than in the control group.

From the foregoing it is evident that photosynthesis plays a substantial role in the synthesis of grain dry matter during maturation, and if it is disrupted in the course of a dry period even at this late stage there may be a strong reduction in yield.

Finally, going back to the experiments on drought, varietal differences should be mentioned. Both varieties were subjected to a five-day drought period at the same stage of grain development, the stages not coinciding in

time, however. Nevertheless climatic conditions during the drought period were almost the same for both varieties, and this permits comparison. It is known that the variety Moscow is less drought resistant than Lyutestsens 62. This is borne out by the greater reduction in grain weight in Moscow than in Lyutestsens 62. Moreover, the rate of grain maturation in the former variety increased to a lesser extent (or did not increase at all), the weight of the vegetative mass of the plant decreased to a greater extent, the percentage of nitrogen in the grain increased to a greater extent, and the grain as well as the vegetative portions lost water to a larger degree. In order to be sure that these are really measures of drought resistance, however, it is necessary to perform similar experiments with a larger number of varieties.

SUMMARY

1. When a dry period occurs during grain maturation, the grain loses water to a smaller extent than the vegetative parts.

2. Initially soil moisture deficiency causes an increase in the rate of grain maturation, or at least not affect it. This increase is at the expense of a preliminary breakdown of the vegetative portions.

3. A soil moisture deficiency initially stimulates the movement of nitrogenous materials into the grain, not only relatively but also absolutely.

4. Photosynthesis occurring during grain maturation (milk stage) plays a substantial part in the accumulation of grain dry matter. The most important cause of the yield reduction resulting from drought at this time is, in addition to the intensified loss of organic compounds in respiration, the decrease in photosynthetic activity.

5. The translocation of nitrogen from the vegetative organs is essentially complete even in the dark.

6. A more drought resistant variety subjected to drought during the period of grain maturation is characterized by a greater increase in the rate of maturation, a smaller loss of water by the vegetative mass, and a less marked increase in the percentage content of nitrogen in the grain.

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WATER CONTENT AND MATURATION OF SEEDS

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Water content of plant seeds is often determined during a study of various aspects of their physiology and biochemistry. The data obtained are used mainly in the calculation of quantities of various substances on a dry weight basis and only sometimes are they used as criteria of the condition of the seeds. But even in this case the data do not ordinarily embrace the entire period of seed development; absolute values of water which provide a picture of seed water balance are rarely given.

Only in a few studies has the subject of seed water regime been gone into in more detail. Thor and Smith [1], for example, describe ripening cotton seeds in terms of their relative and absolute water content as well as in terms of other factors. In Byvshikh's dissertation [2] are presented values for the relative water content of watermelon seeds which indicate that as the seeds ripen it steadily decreases, and only in seeds of overripe fruits does it once again sharply increase. The moisture level of wheat grains [3] falls during ripening more than five-fold; during the first six weeks of grain development it rises, however, and then falls abruptly. Pontovich [4] with poppy, Ryshkovskii and Malysheva [5] with sunflower, Lambou, Parker and Carns [6] with cotton, and Prokof'ev and Novitskaya [7] with poppy and flax give quite a complete description of the water content of seeds during their maturation. Even in these studies, however, the water content was determined with purely secondary purposes. The regular pattern of change in seed moisture content during seed ripening compels the assumption that the degree of seed hydration is directly related to seed condition. It is therefore of interest to make a more detailed and systematic study of seed water balance. It is possible that such a study could reveal a specific correlation between the water content of seeds and the contents of various substances which are an index of the degree of ripeness. In addition to an analysis of these problems, the purpose of our investigations was to study changes in seed water content associated with ripening in relation to those changes which are induced by changing internal and external factors.

METHODS

The plant used in these studies was oil poppy. The studies were made at the All-Union Research Institute of Olive Crops and Essential-Oil-Containing Crops–(VNIIMÉMK, Krasnodar) in 1957-1958. Each sample consisted of 30-50 poppy capsules of the same age. The moisture content of the seeds and the capsule was determined by drying to constant weight; young seeds were dried in a thermostat at 50-60° for 1-2 hours and older seeds were dried at 70-75° for 2-5 hours.

For determination of absolute weight five aliquots of 1000 seeds each were dried to a constant weight at the same temperature. The average of the five aliquots was used for calculation of absolute water content and degree of seed hydration. For analytical purposes seeds removed from the capsules were fixed with live steam for 15-20 minutes and subsequently dried in a ventilated oven or in a thermostat at 40° to the air-dry condition.

Fat content was determined at the biochemical division of VNIIMÉMK using the standard extraction procedure. Carbohydrates were separated chromatographically in a butanol-acetic acid- water (5 : 1 : 4) mixture for 2.5 days, after which the quantity was determined colorimetrically by the phenol method of Dubois et al [5].

Measurements of the relative humidity inside the capsules were made with a specially constructed hygrometer based on an electrolytic principle and adapted for measurements in a small volume of air. A description of devices of this type has been published [9-11].

EXPERIMENTAL RESULTS

Results of determinations of seed moisture content are presented in Fig. 1. The change in relative water content of ripening poppy seeds follows a regular course; during the first days after flowering the percentage water content increases somewhat, after which there is a gradual decline for almost the entire period of seed formation. Just shortly before complete maturity there is an abrupt decrease in water content which is followed by stabilization at about 5-6%.

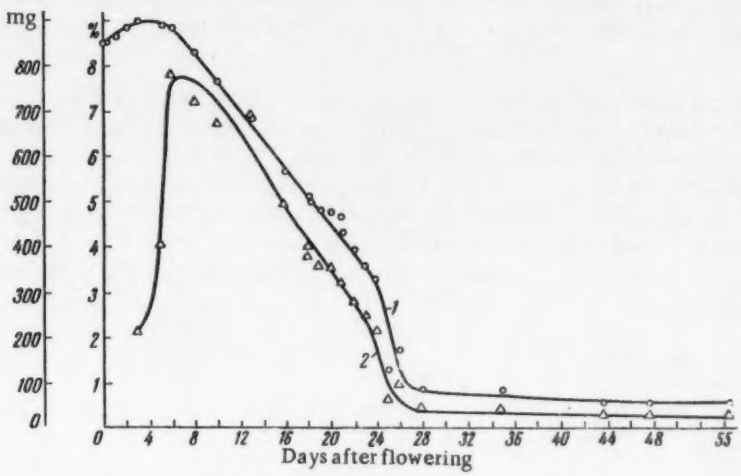

Fig. 1. Moisture content of poppy seeds during ripening.
1) Relative moisture content of seeds (in %; each unit corresponds to 10%);
2) absolute moisture content of seeds (in mg per 1000 seeds).

The picture is different with respect to absolute water content (Fig. 1, 1). There is a striking increase in water content in the first days of seed development followed by rapid water loss almost to the stage of complete maturity.

TABLE 1

Change in Seed Composition in Relation to Atmospheric Humidity (in %)

Age of fruits after flowering, in days	Item measured	Initial	After 10 days		Age of fruits after flowering, in days	Item measured	Initial	After 10 days	
			in moist chamber	in the field				in moist chamber	in the field
6	Moisture	88.03	69.24	59.33	10	Moisture	79.46	52.05	47.39
	Fats	20.7	36.2	47.7		Fats	38.7	43.5	48.5
	Glucose	1.40	0.20	0.11		Glucose	0.61	0.09	0
	Fructose	3.90	0.51	0.24		Fructose	0.98	0.14	0
8	Moisture	87.30	—	—	11	Moisture	75.48	—	46.67
	Fats	31.0	36.4	48.7		Fats	43.0	46.5	48.3
	Glucose	1.00	0.16	0		Glucose	0.63	0	0
	Fructose	1.11	0.33	0		Fructose	0.75	0.09	0

The curves for water content of ripening poppy seeds are similar in their main features under varying weather conditions, although individual stages may be markedly prolonged or curtailed. For example according to data obtained in this laboratory, in cold, wet years the ripening period is prolonged, and the seeds lose water at a considerably slower rate. In hot, dry years, on the contrary, seed formation and seed dehydration are more rapid. It seemed important to establish to what degree external conditions which determine the rate of seed drying are reflected in the degree of seed maturity. To answer this question, the following experiment was designed. Seed capsules 6-11 days after flowering were enclosed in transparent perforated chambers so that the humidity surrounding the capsules was about 100%. The chambers were ventilated twice a day. Plants on the same plot not enclosed in chambers served as controls. The experiment lasted ten days; results are presented in Table 1.

As Table 1 shows, exposure of the seed capsules to a moist atmosphere had a definite effect on all the items measured, which serve as criteria of seed maturity. Moisture content of seeds from experimental plants was higher than that of seeds from control plants, i.e. the rate of dehydration was decreased. Oiliness of the seeds — an index of their maturity — was also substantially reduced in a moist atmosphere. The behavior of monosaccharides is also indicative. Preliminary investigations had shown that the content of monosaccharides (glucose and fructose) decreases as the seed ripens, and in ripe seeds they are absent. The data presented indicate that enclosure of capsules in damp chambers impedes the disappearance of glucose and fructose.

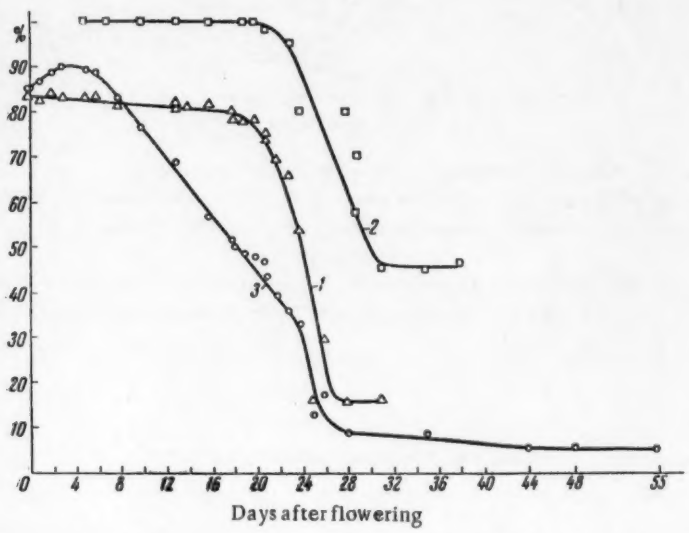

Fig. 2. Moisture content of tissues of poppy capsules and of the internal atmosphere (in %).
1) Moisture content of capsular tissues; 2) moisture content of air inside capsules; 3) moisture content of seeds (given for comparison).

Thus, the data obtained are fully consistent in indicating that conditions which inhibit water loss also impede conversions characteristic of the ripening process (accumulation of fats, disappearance of monosaccharides).

In order to define the conditions in which poppy seeds develop in nature, the water content of the capsule tissues and the relative humidity inside the capsules were determined (Fig. 2). The curves for the change in moisture content of the capsule during fruit development show that during the main period of its formation the water content of the tissues declines very little (3-4%). For several days subsequently, however, there is a very rapid loss of water by the capsules which is apparently related to a slowing up and possibly even a cessation of the flow of water into them. This is indicated by the gradual yellowing and drying out of the pedicels, and the subsequent withering of the whole plant. The water content in the capsules is gradually stabilized at a very low level (14-16%).

Measurements of the relative humidity inside the capsules show (Fig. 2, 2) that it remains close to the saturation value (about 100%), up till the beginning of a perceptible drying of the capsules. An unsaturated atmosphere was encountered first at the time the water content of the fruit tissues had begun to fall, when it was at 73-75% (in the 1957 experiments, on the 21st day after flowering). A further decline in the internal relative humidity is somewhat retarded because of the decrease in water content of the capsular tissues. At the time the capsules have lost the maximum amount of water (water content 16%), the relative humidity inside them is stabilized at a level which approximates that of the external air (45-47% in the capsule and 35-42% in the surrounding atmosphere).

Thus, the critical moment in seed development, which is characterized by an abrupt decrease in both relative and absolute water content, coincides with the drying out of the fruit and the reduction of moisture content of its internal atmosphere. The cessation of water flow to the seeds following their abscission from the placenta and the unsaturated condition of the internal atmosphere promote the rapid loss of water from the seeds. The pattern of water loss is determined under these conditions mainly by the moisture content of the surrounding environment.

Our data may therefore be compared with values of the so-called equilibrium moisture content of seeds, i. e. the percentage content at a definite relative humidity. Curves for the eqiulibrium moisture content of mature oil-bearing seeds have been given by Larmour, Sallans and Craig [12] which show that at a relative humidity of 43% the water content of sunflower seeds is 6.3% and of flax seeds 6.4%. According to Sharoiko [13], sunflower seeds at 45% relative humidity contain about 5.5% water. The values we have obtained with poppy seeds in field conditions are in good agreement with published values: when the relative humidity inside the fruit is stabilized at 45-47%, the moisture content of seeds after a gradual decline is stabilized at 5-6%.

Only a small part of the water entering the seeds is lost in this manner, however – not more than $\frac{1}{8}$ - $\frac{1}{4}$ of its maximal content in the seeds (see Fig. 1, 2). The actual amount of water passing through the seeds is considerably higher than that determined in our experiments because there are two opposing processes taking place throughout the period of seed formation – water uptake and water loss. The absolute water content in the seeds is the resultant of these processes, and a change in water content during seed development indicates whether water uptake is more rapid than water loss or the reverse. Of particular interest is the loss of significant amounts of water by seeds in a saturated atmosphere. This is shown by data presented previously (Table 1) as well as by the following experiment. Seeds taken from freshly collected poppy capsules on the 14th day after flowering were placed in glass chambers in one of which there was a container with anhydrous calcium chloride and in the other – a container with water. A comparatively low humidity was maintained in the first chamber by the regular renewal of calcium chloride (one or two times a day), while in the second the atmosphere was constantly saturated as shown by the fact that there were droplets of moisture on the chamber's walls. Four days later the moisture content of the seeds in the chambers and of seeds taken from the field at the end of the experiment was measured.

The following values were obtained (in %):

Initial		68.57
Final	dry chamber	36.58
	moist chamber	54.07
	field plants	53.52

A comparison of these values shows that isolated seeds in a saturated atmosphere continue to lose water. It is also apparent that the extent of this loss approximates that which is observed in seeds of the same age from plants in the field. Seeds kept in the dry chamber underwent a marked water loss in distinction to seeds under natural conditions. This is additional proof that under natural conditions water loss by young seeds occurs at very high moisture levels.

The important role of fruit age in seed dehydration is confirmed by experiments in which, instead of isolated seeds, whole capsules are placed in chambers. Differing humidities were maintained by the same method, i. e. by water and calcium chloride; it was necessary to use considerable amounts of the latter and to renew it three or four times a day in order to prevent, from the outset, the appearance of moisture droplets on the walls of the chamber. The results of these experiments are summarized in Table 2.

TABLE 2

Loss of Water by Seeds and Capsules in the Field and after Their Exposure for Six Days to Various Humidities (moisture content of seeds and capsules, in %)

Age after flowering, in days	Seeds				Capsules			
	initial in field	in dry chamber	in damp chamber	on plants in the field	initial in field	in dry chamber	in damp chamber	on plants in the field
14	68.57	15.37	52.88	48.22	80.96	31.71	82.22	78.45
18	51.96	12.45	43.92	18.39	78.04	17.39	72.39	27.17
25	35.71	4.21	24.26	8.03	53.65	9.18	29.14	14.14

The results show that fruits of all ages studied, from comparatively young to almost mature, rapidly lose water under dry conditions both from the capsules and the seeds. Under natural conditions this is prevented mainly by uptake of water from outside. In a damp chamber young seeds lose water to a considerable extent which approximates the water loss under normal field conditions (16% and 20%). At a more mature stage, water loss in a damp chamber is considerably less than under natural conditions (at 18 days, 8 and 34%; at 23 days, 11 and 27%). From these data it is concluded that a high moisture content of the surrounding atmosphere does not exert a marked effect on the development of young seeds. On the contrary, these same conditions clearly impede the maturation of older seeds, which is consistent with the natural pattern of change of moisture content in the internal atmosphere of the fruit.

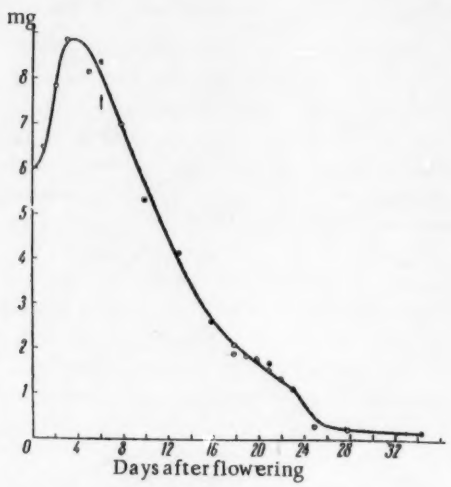

Fig. 3. Moisture content of hydrophilic materials of poppy seeds (in relative units). Arrow indicates moment of fat appearance.

In a saturated atmosphere, evaporation from fruits is extremely limited, and therefore young poppy seeds lose water not by this method but by some other method. In a study by Skripka [14] it was shown that under severe climatic conditions water from watermelon fruits flows into the plant's vegetative organs. This, however, does not mean that there was a flow of water from the seeds; moreover, a previously described experiment in which the presumed outflow of water from the seeds was excluded by the fact that the seeds were isolated, but in which water loss continued to occur in a saturated atmosphere, shows that other paths of water loss exist.

Several paths of water loss by young seeds may be suggested: the utilizations of water in synthetic processes —here water disappears as a chemical compound, but its component elements remain in the seed; water loss against a moisture gradient or "squeezing out" as a result of decrease in cellular hydrophily, and increase in hydrophobic materials. In connection with the latter, it was assumed that dehydration of seeds is in some measure related to the accumulation of fats. This assumption takes on more validity in light of the fact that the moment of decrease of water in seeds corresponds with the beginning of fat accumulation. Another picture is obtained, however, if the quantity of water is related to a unit of the hydrophilic part of the seed. Such data were obtained by calculations involving the weight of the seeds, the degree of their hydration, and the amount of fat (Fig. 3).

Figure 3 shows that in the first day of seed development their water content increases, i. e. more water per unit weight is retained. Later this capacity falls rapidly, and the decline in the hydration of the hydrophilic portion of the seed (third day after flowering) for several days outstrips the appearance of fat. Consequently, a change in the water retaining capacity of the seeds should be related not so much to the appearance of fats as to

the change in the colloidal-chemical properties of the hydrophilic materials in the seed. The investigation of Blagoveschenskii [15], Genkel' [16] and Oknina [17] suggest that such changes may occur during stratification or during transition to the dormant conditions.

SUMMARY

The relative and absolute contents of water in ripening seeds vary in a regular fashion. External conditions (moisture content of the air) determine the rate at which the seeds dry up but do not alter the general trend of change. The water content of seeds can be employed as an objective index of their degree of maturity; an expression of the latter is the disappearance of monosaccharides and accumulation of fat. There is a direct relationship between the water content of seeds and the high moisture content of the capsule as well as the high atmospheric humidity inside the fruit during seed development; a sharp drop in these indices during the late stages of fruit ripening is also correlated with seed water content.

Loss of water by seeds can be divided into physical losses (evaporation) and biological losses (active transfer in a saturated atmosphere, passive "squeezing out" due to decrease in hydrophily, utilization in metabolic processes). The purely physical loss of water plays a decisive role only during the last stages of ripening. Most of the water is lost by biological means.

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PHYSIOLOGICAL CHANGES IN FRUIT TREES DURING CHEMICAL DEFOLIATION

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The necessity for chemical defoliation of fruit trees has been shown previously, and the corresponding practical recommendation had been made [1]. As to the physiological changes which occur, this phase of the problem had been little clarified. However, it should be noted that there is a great deal of information in the literature relative to the physiology of dropped leaves in artificial defoliation of other plants, as well as natural falling of leaves in various plants [2-6].

In this report we present the results of our investigations in clarifying certain physiological changes occurring in fruit trees upon their defoliation.

The study was conducted in the K.A. Timiryazev Institute of Plant Physiology AN SSSR from 1955 to 1958 inclusive. The objects of investigation were 2-and 3-year old saplings of Pepin Shafrannyi apple trees, replanted from the nursery into a vegetative tub of 8 kg soil capacity.

Spraying of plants by defoliant solutions was conducted as follows: one-year old saplings – 1000 l/hectare and two – and three-year old – 2000 l/hectare.

It should be noted that a well-timed treatment of plants (at the end of August and the beginning of September) by 0.25% magnesium chlorate hexahydrate [$Mg(ClO_3)_2 \cdot 6H_2O$] and 0.075% endothal (disodium salt of 3,6-endoxohexahydrophthalic acid) causes a dropping of all leaves in 12-15 days.

Stronger or weaker concentrations do not produce the desired effect. With stronger concentrations a desiccation of leaves occurs without forming separation layers, and with weaker ones–the usual course of physiological processes is only slightly distrubed and the leaves do not drop.

The data in Table I show that the greater the concentration of magnesium chlorate and endothal, the weaker is the process of chlorophyll destruction. At the optimum concentration of magnesium chlorate (0.25-0.5%) and endothal (0.075-0.1%) for drop-off of leaves, the chlorophyll destruction proceeds with the greatest intensity.

Besides the destruction of chlorophyll, the effect of defoliants on photosynthesis and respiration was checked. The recording of these processes was conducted on the same leaf sample; first the respiration intensity was determined (60 minute exposure), and then the photosynthesis (10 minute exposure). Determination was conducted by the Warburg apparatus at 25°. Carbonate buffer No. 9 was used as a source of carbonic acid. Photosynthesis was calculated at an illumination intensity of 22,000 lux (Table 2).

As shown in Table 2, defoliants markedly diminish photosynthetic intensity. The effect of magnesium chlorate and endothal proved to be somewhat different: in plants treated by magnesium chlorate the photosynthesis is almost totally restored 24 hours after treatment, and in subsequent days it weakens markedly and ceases completely on the fourth day; in plants treated by endothal, the photosynthesis diminishes continuously, but is not wholly suppressed.

The data obtained (Table 3) show that in depressing photosynthesis, defoliants intensify respiration, and the more the latter is intensified, the more the photosynthesis is suppressed.

TABLE 1

Effect of Magnesium Chlorate and Endothal on Chlorophyll Content (treatment on September 11, 1957; chlorophyll was extracted by ethyl alcohol and was measured in the extract spectrophotometrically)

Experimental variant	Chlorophyll content, in mg/g dry weight of leaves					
	September 14			September 17		
	a	b	Total	a	b	Total
Control	5.50	1.93	7.43	4.04	1.54	5.58
Magnesium chlorate, %:						
0.125	38.7	1.47	5.34	3.11	1.34	4.45
0.25	3.40	1.12	4.52	3.08	1.25	4.33
0.50	3.74	1.32	5.06	3.03	1.33	4.36
0.1	4.46	1.59	6.05	3.02	1.43	4.45
Endothal, %:						
0.075	3.11	1.12	4.23	2.38	1.06	3.43
0.125	3.16	1.35	4.51	2.53	1.12	3.65
0.25	3.40	1.32	4.72	2.77	1.22	3.99
0.5	3.57	1.38	4.95	2.78	1.25	4.03
1.0	4.19	1.54	5.73	3.35	1.38	4.73

TABLE 2

Effect of Defoliants on Photosythetic Intensity (treatment of August 18, 1957)

Experimental variant	Intensity of O_2 photosynthesis, in μl/hour / m^2 of leaf area				
	August 18	August 19	August 20	August 21	August 22
Control	2169	2289	2044	2108	2114
0.25% magnesium chlorate	1511	2102	780	0*	0*
0.075% endothal	1635	1436	602	508	430

*Photosynthesis was totally supressed, and absorption of 505 μl per hour August 21 and 650 μl per hour August 22 was noted.

Studying the effect of defoliants on types of respiration in apple tree leaves (by the method described by Rakitin, Povolotskaya and Sedenko [7]), we found that the total activity of oxidative enzymes is increased by the action of magnesium chlorate and endothal, and that this increase is due basically to the increased activity of flavine enzymes, and, finally, that the degree of participation of both copper and iron-containing enzymes in the respiration process is weakened.

A somewhat different picture is observed in apple tree shoots: the activity of oxidative enzymes immediately after treatment is increased somewhat, but then decreases and remains at almost the same level as in the shoots of untreated plants; the second day after treatment a doubling of activity of copper-containing enzymes is observed; the activity of iron-containing enzymes also is almost doubled, but the proportional share of these enzymes in the total respiration remains the same as in the untreated plants. Defoliants cause a notable diminution of flavine enzyme activity in the shoots, while magnesium chlorate causes irreversible suppression of flavoenzymes, although endothal suppresses their activity during the first few days and increases it subsequently.

TABLE 3

Effect of Defoliants on Intensity of Photosynthesis and Respiration (treatment August 18; determination August 21)

Experimental variant	$\mu l\ O_2$/ hours/ m^2	
	Photosynthesis	Respiration
Control	2108.0	1583.0
0.25% magnesium chlorate	0*	2738.0
0.075% endothal	508	2125.0

* Photosynthesis was completely suppressed and O_2 absorption was observed to be 505 μl per hour.

TABLE 4

Effect of Magnesium Chlorate and Endothal on Carbohydrate Content of Leaves (mg/ g dry weight) (treatment August 18, 1957; carbohydrate determination conducted by Bertrand method)

Experimental variant	August 19			August 20			August 22		
	Monosac-charide	Disac - charide	Starch	Monosac-charide	Disac - charide	Starch	Monosac-charide	Disac-charide	Starch
Control	10.96	27.93	14.02	10.71	36.27	13.64	16.63	51.84	16.51
0.25% magnesium chlorate	13.48	49.15	21.03	28.07	26.49	10.15	10.45	9.75	6.28
0.075% endothal	20.53	12.19	10.14	13.63	12.7	11.29	14.38	11.92	8.78

TABLE 5

Effect of Magnesium Chlorate and Endothal on Phosphorus Metabolism of Leaves (mg per g, dry basis) (treatment August 18, 1957, 1 g dry weight; determination of phosphorus by Fiske-Subbarow)

Experimental variant	August 19			August 20			August 22		
	Organic	Inor-ganic	Total	Ogranic	Inor-ganic	Total	Organic	Inor-ganic	Total
Control	0.80	0.31	1.11	0.86	0.38	1.24	1.22	0.25	1.47
0.25% magnesium chlorate	0.50	0.54	1.04	0.43	0.40	0.83	0.36	0.16	0.52
0.075% endothal	0.65	0.40	1.05	0.46	0.52	0.98	0.39	0.17	0.56

We also recorded changes in the type of leaf respiration due to action of different doses of magnesium chlorate (solutions in concentrations of: 0.25%, 0.5%, 0.1% and 2.0%). In almost all cases a noticeable increase in total activity of oxidative enzymes was observed, and this increased activity was maintained until the drop-off of leaves. The greater the magnesium chlorate concentration, the higher was the activity of flavine enzymes. The over-all activity of copper- and iron-containing enzymes was also lower in treated plants; with the increase in magnesium chlorate concentration there was a noticeable diminution in activity of these enzymes.

As our analyses have shown (Table 4), comparable defoliants affect carbohydrate metabolism of leaves in different ways: the second day after treatment magnesium chlorate greatly increases the content of disaccharides and starch; endothal considerably increases content of monosaccharides. Subsequently a decrease in starch and disaccharides is observed in both cases. At the time of separation from the plant, the leaves of the treated plant are greatly impoverished in monosaccharides, disaccharides, and starches, while the content of cellular tissues is practically unchanged.

The data indicate that the defoliants also substantially change the phosphorus metabolism (Table 5). The total phosphorus decreases insignificantly 24 hours after treatment, while the inorganic phosphorus increases. The latter occurs, evidently, because of the decomposition of organic phosphorus compounds (hydrolysis). By the third day from the time of treatment of plants by magnesium chlorate, the total phosphorus in the leaves is markedly reduced. The inorganic phosphorus also decreases at this time, but it proves to be higher than in the control plants. At the time of leaf dropping the content of all forms of phosphorus (total, organic, and inorganic) in the leaves decreases.

Our studies have shown that the action of magnesium chlorate and endothal causes marked shifts in nitrogen metabolism of the leaves. The first few days after treatment an accumulation of inorganic nitrogen occurs and at the time of defoliation a noticeable decrease is observed in all identifiable forms of nitrogenous substances (total nitrogen, protein, organic, and inorganic nitrogen).

TABLE 6

Effect of Magnesium Chlorate on the Flow of P^{32} from Leaves of Pepin Shafrannyi Apple-Tree Seedlings (treatment September 28, 1957)

Experimental variant	Leaf position	P^{32} content in pulses per g leaf dry weight			
		Before treatment	Days after treatment		
			2nd	5th	7th.
Control	Upper	909.0	896.6	859.5	919.9
	Middle	1875.0	1781.3	1668.7	1687.5
	Lower	2431.0	2399.4	2284.2	2253.5
0.25% magnesium chlorate	Upper	1000.0	950.0	890.0	850.0
	Middle	2800.0	2520.0	1680.0	1638.0
	Lower	2900.0	2667.0	1972.0	1887.9

In our experiments the effect of defoliants on the outflow of phosphorus from leaves was also studied. The effect was calculated by using $Na_2HP^{32}O_4$ in the experiments. A solution of this compound, with a specific activity of 9.9 μC, was introduced into sapling plants through a strand of roots which was immersed into a solution of the preparation [8] for this purpose. Such immersion was conducted for 2 days before treating the plants with magnesium chlorate. The flow was studied on leaves of the first, second, and third shoot, counting form the top of the plant. In these experiments leaves of different layers were taken (the upper, middle, and lower). The phosphorus flow was calculated from alteration of leaf radioactivity after the plants were sprayed by a solution of magnesium chlorate. For calculation of radioactivity half-leaves were used. As shown in Table 6, a considerable flow of P^{32} from leaves is observed even by the second day after treatment. The largest phosphorus flow occurred the fifth day after treatment; then the flow nearly ceased. The phosphorus flow from leaves of different layers was unequal: the phosphorus flow was greater from leaves of the middle layer than from leaves of the upper and lower layers. Also the phosphorus flow was unequal from leaves of control plants; the phosphorus outflow from leaves of the middle layer was greater than from leaves of the lower layers. The phosphorus flow was more intense from leaves of treated plants than from the controls.

SUMMARY

Physiological changes in fruit tree saplings were produced as a result of fall defoliation with magnesium chlorate hexohydrate $Mg(ClO_3)_2.6H_2O$ and endothal (disodium 3,6-endoxohexahydrophthalate), which are efficient means of accelerating the falling of leaves in fruit trees.

Defoliant sprays which accelerate falling of leaves produce important changes in the metabolism of the leaves. Thus, chlorophyll is destroyed, photosynthesis inhibited and the total general activity of oxidative enzymes increased. Furthermore a significant increase of the activity of flavin enzymes and weakening of the activity of iron and copper - containing enzymes is observed and respiration is greatly stimulated; hydrolysis of starch and phosphorus organic compounds is intensified and the content of sugars and phosphorus compound is lower. Finally, the flow of metabolites is found to be higher.

The data obtained indicate that underlying the physiological action of defoliants is a significant violation of metabolism which exhibits itself in a pronounced weakening of the synthetic activity of leaves and intensification of disintegration processes in them.

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BRIEF COMMUNICATIONS

EFFECT OF CONDITIONS OF NITROGEN-PHOSPHORUS FEEDING ON DEVELOPMENT AND PHOSPHORUS METABOLISM OF SUMMER WHEAT SEEDLINGS

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In the present study the relationship between phosphorus and nitrogen feeding of summer wheat seedlings of the Moscow variety was investigated. By use of the radioactive isotope of phosphorus (P^{32}) a study was conducted of phosphorus absorption by seedlings from a nutrient solution in relation to the degree with which the seedlings were provided with nitrogen and phosphorus. In order to obtain a clearer picture of this relationship, the seedlings were first (for 7 days) sprouted on solutions without nitrogen or phosphorus, or without both nitrogen and phosphorus, which permitted their impoverishment in these elements in a deficient medium. The seedlings were then transferred to complete nutrient mixtures with labeled phosphorus and were sprouted in these for another 7 days.

Nutrient mixtures were used prepared on the basis of a Pryanishnikov mixture.

To maintain pH uniformity in the nutrient solutions, these were periodically replaced by previously and simultaneously prepared solutions of each variant. This also provided a uniform relationship between stable and radioactive phosphorus isotopes in solution, independent of solution change.

In these seedlings, the fractional content of phosphorus obtained from solutions labeled by the radioactive indicator and the total phosphorus content* were determined. The Kursanov [1] and Sokolov [2] methods were used. The separation of inorganic from acid-soluble organic phosphates was conducted by extraction with isobutyl alcohol [3].

Elimination of nitrogen or phosphorus from the nutrient medium was unfavorable to seedling development during the first 7 days of cultivation (see Figure). Absence of phosphorus in the medium had a negative effect on rootlet development; absence of nitrogen, on shoot development.

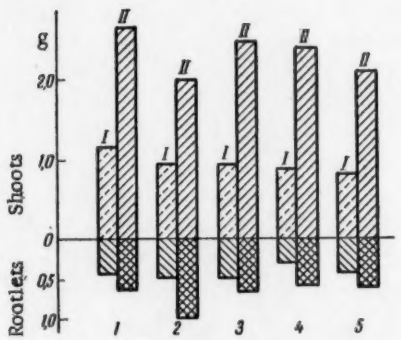
Determination of total nitrogen and total sugar content in shoots of 14-day-old seedlings showed that shoots of nitrogen-less variants are very considerably impoverished in nigrogen, while at the same time they are considerably richer in sugars than the shoots of seedlings of other variants (Table 1).

Based on the studies of Pryanishnikov [4] on the relationship of nitrogen absorption to presence of carbohydrates, and of Kursanov [5] on movement of substances within the plant, the rise in rootlet growth with nitrogen deficiency can be explained by the fact that sugars flowing into the roots were not used for nitrogen fixation after glycolytic decomposition, and could to a larger degree be used in building cellular tissue, hemicelluloses, and other compounds in the roots.

Differences in metabolism brought about by an unequal supply of nitrogen must, doubtless, also affect phosphorus absorption from the medium. The data obtained (Table 2) confirm this. Seedlings on a full nutrient mixture absorbed more phosphorus. Seedlings poor in nitrogen absorbed considerably less phosphorus than seedlings which were fed with nitrogen.

* By total content is meant the total phosphorus content, including the labeled as well as that found in the plant before feeding with labeled phosphorus.

Seedlings placed, during the first 7 days of cultivation, on a mixture deprived of phosphorus were distinguished by the most intense absorption of labeled phosphorus. These data correspond with data of Tueva [6] obtained on older squash plants. Seedlings simultaneously deprived of nitrogen and phosphorus absorbed phosphorus consierably less intensely.



Effect of nutrient conditions on development of summer wheat seedlings. Dry substance (in g) calculated per 100 seedlings: I) shoots and rootlets of 7-day-old seedlings; II) 14-day-old; 1, 2, 3, 4, 5), experimental variants (see Table I).

Determination of phosphorus compound fractions (Table 3) showed that the intensity of phosphorus absorption is directly dependent on the organic phosphorus content. In this connection, a change in phosphorus content is found in all the determined fractions of a given variant. With nitrogen deficiency, the phosphorus content of all organic phosphate fractions is diminished, just as there is an increase in phosphorus content in all fractions when absorption of nitrogen is intense. However, the broades range in labeled phosphorus content among the variants is observed in the nucleoprotein fraction.

Separate determinations of total phosphorus and phosphorus labeled or absorbed from the solution during the experimental period permit the tracing of the replacement of each fraction by labeled phosphorus (Table 4).

Table 4 indicates the relatively similar data for different fractions of a given variant in the shoots or rootlets. When there is a decrease in the proportion of total absorbed phosphorus, there is also a decrease in the proportion of labeled phosphorus in the given variant.

TABLE 1

Effect of Nutrient Conditions on Nitrogen and Sugar Content in Shoots of Summer Wheat Seedlings

Variant no.	Nutrient conditions*	Total nitrogen			Total sugar %
		in %	in mg per seedling	Absorption from solution, mg per 100 seedlings	
1	Full mixture/full mixture	5.12	138	+83	3.41
2	Without N/ without N	2.69	55	0.0	11.29
3	Without N/ full mixture	5.21	129	+74	3.05
4	Without P/ full mixture	5.63	136	+81	3.18
5	Without NP/ full mixture	5.12	110	+55	3.33

* The numerator – first 7 days; the denominator – following 7 days.

However, if different fractions are compared as to degree of enrichment with labeled phosphorus, then one is struck primarily by the relatively high share of absorbed phosphorus in the nucleoprotein fraction in the rootlets which, as we see it, emphasizes the importance of nucleoproteins in phosphate absorption, as noted by other authors [7, 8].

TABLE 2

Phosphorus (P_2O_5) Content of 14-Day Old Summer Wheat Seedlings in Relation to Nutrient Conditions

Variant No.	Nutrient conditions	In shoots		In rootlets	
		Total	Labeled	Total	Labeled
1	Full mixture/ full mixture	57.1	27.9	10.3	5.9
2	Without N/ without N	41.5	15.5	13.3	5.9
3	Without N/ full mixture	50.5	24.2	9.5	4.9
4	Without P/ full mixture	47.5	32.1	9.2	5.7
5	Without NP/ full mixture	36.1	20.8	8.6	4.7

TABLE 3

Distribution of Absorbed Phosphorus by Fractions in 14-Day-Old Summer Wheat Seedlings in Relation to Nutrient Conditions

Variant No.	Nutrient conditions	Percent P_2O_5 in dry substance by fractions					Inorganic P_2O_5 in % of total
		Total phosphorus	Nucleoproteins	Phosphatides	Acid-soluble organic compound	Inorganic phosphates	
	In shoots						
1	Full mixture/ full mixture	1.039	0.132	0.114	0.087	0.748	72.0
2	Without N/ without N	0.758	0.059	0.072	0.040	0.609	80.5
3	Without N/ full mixture	0.975	0.142	0.119	0.070	0.620	63.5
4	Without P/ full mixture	1.337	0.203	0.173	0.120	0.900	67.0
5	Without NP/ full mixture	0.975	0.167	0.135	0.065	0.585	60.0
	In rootlets						
1	Full mixture/ full mixture	0.941	0.211	0.124	0.132	0.477	50.7
2	Without N/ without N	0.594	0.087	0.067	0.063	0.387	65.2
3	Without N/ full mixture	0.743	0.160	0.098	0.093	0.392	52.8
4	Without P/ full mixture	0.972	0.253	0.132	0.149	0.478	49.2
5	Without NP/ full mixture	0.774	0.179	0.110	0.111	0.372	48.1

The higher proportion of labeled phosphorus in the fraction of acid-soluble organic compounds and nucleoproteins in rootlets reflects the relationship of phosphorus absorption to respiration and synthesis of acids – acceptors of the absorbed nitrogen [9]. This relationship becomes very evident on comparison of data on the nitrogen-free variant with data on other variants. Absence of nitrogen in the nutrient medium during the experimental period, which removes the necessity of intense acid synthesis, lowered the proportion of labeled phosphorus considerably in the fraction of acid-soluble organic compounds in the rootlets. Thus, only in the rootlets of the seedlings of this variant was the share somewhat lower (38.8%) than the sum of absorbed phosphorus in the total content (44.3%). In the rootlets of the seedlings of all other variants the reverse is noted: the proportion of labeled phosphorus in the acid-soluble organic compound fraction is larger than in the total.

In the variant with phosphate starvation the seedlings at the start of the experiment were depleted of phosphorus. In these, to a larger degree than in the seedlings of other nutritive variants, transference of phosphorus from the rootlets to the shoots predominated. The synthesis of acid-soluble organic phosphorus compounds, where the intermediate products of carbohydrate-phosphate metabolism are included, proceeded principally by utilization of the absorbed phosphorus. As a result, this fraction in the rootlets was enriched by labeled phosphorus to the extent of 71.6%. At the same time the inorganic phosphate fraction in the rootlets was enriched with labeled phosphorus by only 59.0%.

TABLE 4

Phosphorus Metabolism for a 7-Day Period in Fractions of Different Phosphorus Compounds

Variant No.	Nutrient conditions	Share of labeled phosphorus in % of total content in fractions				
		Total phospho-rus	Nucleo-proteins	Phospha-tides	Acid-soluble organic com-pound	Inorganic
	In shoots					
1	Full mixture/ full mixture	48.7	41.9	42.0	32.7	59.7
2	Without N/ without N	37.3	30.2	32.0	22.3	42.0
3	Without N/ full mixture	48.2	46.1	41.4	31.3	52.5
4	Without P/ full mixture	67.8	60.2	59.4	46.5	84.0
5	Without NP/ full mixture	57.7	49.7	52.9	34.7	59.9
	Mean	51 9	45.6	45.5	33.5	59.6
	In rootlets					
1	Full mixture/ full mixture	57.4	60.9	52.7	59.8	52.3
2	Without N/ without N	44.3	43.1	35.6	38.8	47.2
3	Without N/ full mixture	51.8	51.5	46.2	51.3	51.0
4	Without P/ full mixture	62.1	68.9	56.1	71.6	59.0
5	Without NP/ full mixture	54.0	55.6	50.9	54.1	49.2
	Mean	53.9	56.0	48.3	55.1	51.6

An opposite relationship was observed in the shoots. The fraction of acid-soluble phosphorus compounds was enriched by 46.5%, while the inorganic phosphate fraction – by 84.0%.

Thus, the degree of enrichment by labeled phosphorus of one or another fraction, as related to physiological characteristics of nutrition, permits a wider concept of the interrelationship of plant nourishment by nitrogen and phosphorus, which is highly significant in formative processes. In this connection it should be noted that the interrelationship referred to is of a general character, and is worthy of greater attention in the study of plant nutrition during the initial period of development, since at this time spike formation of the main stalk begins, which determines the size of the summer wheat crop.

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EFFECT OF WATER SUPPLY ON BIOCHEMICAL CHANGES IN COTTON LEAVES AND SEEDS

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Physiological-biochemical changes occurring in plants in relation to differences in water supply show that lack of moisture in the soil causes a disruption in the plant's metabolism. The decline in yield due to deficiency of water results not only from retardation of growth, but also from rhythm disruption of individual biochemical processes [1, 2].

The aim of this investigation is to study the effect of soil moisture of different degrees under field conditions on biochemical changes in cotton leaves and seeds during ripening. A comparative biochemical study of the cotton plant under irrigation and without irrigation affords a picture of the physiology not only of the irrigated, but also of the nonirrigated cotton plant which covers large areas of the cotton-raising sections of our country.

As indications of the condition of the plant the carbohydrate-protein metabolism, the catalase activity, and the content of ascorbic acid, which accomplishes a number of the more important functions in plant metabolism, were studied. In the cotton seeds the accumulation of oil and protein and of the toxic pigment gossypol were studied.

Five varieties of cotton plant were included in the investigation: 1306 (fast-ripening), 108-F (medium-ripening), S-460 (slow-ripening) of species G. hirsutum L; 2 and 3 – G. barbadense; and K-922 – G. arboreum. Samples of the cotton plant were cultivated on the Mid-Asiatic Experimental Station of the All-Union Institute of Plant Husbandry (near Tashkent) in 2 experimental variants: on an irrigational agricultural background where during the vegetative period the soil moisture was at a level of 65-70 % of full moisture capacity, and on a sector where irrigation was excluded during the period of cotton plant vegetation, with soil humidity of the arable land at 35-40% of full moisture capacity.

Dates for taking leaf samples for analysis during the years of investigation (1953-1954) were coordinated with the basic periods of cotton plant development. The first sample was taken during massive budding, the second during flowering, and the third during fruit formation and ripening (August 16-20).

The lack of irrigation on the nonirrigated sector reacted markedly on cotton growth processes (Fig. 1) and led to a diminution of the general assimilative surface of the shrub. On the sector without irrigation the growth of the main stalk ceased almost completely in all cotton plant varieties by July 20 (flowering). Retardation of growth processes was also evidenced by the insignificant gain in weight of leaves during the vegetative period. Changes in the complex of background conditions also changed the tempo of cotton plant development.

Changes in growth and development of the cotton plant dependent on a sufficient moisture supply are related to changes in the biochemical processes. The difference in accumulation of dry substance in the leaves of irrigated and nonirrigated cotton plants should be noted. Depending on the variety and the phase of development, the quantity of dry substance in the leaves was: with irrigation 22.5-27.8% without irrigation 24.0-33.8 %.

In the cotton leaves, the major portion of sugars consists of monosaccharides. Their maximum accumulation is found in the phase of 100% budding (with irrigation 2.34-3.82 %, without irrigation 2.40-5.53%). An increased sugar content was noted in the leaves of nonirrigated cotton plants during the entire period of vegetation.

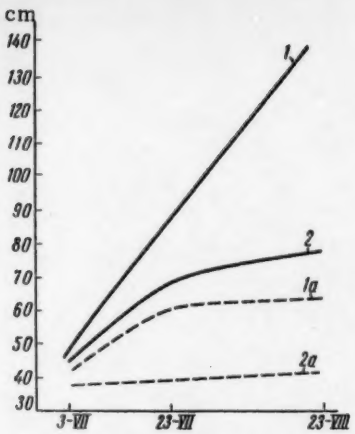

Fig. 1

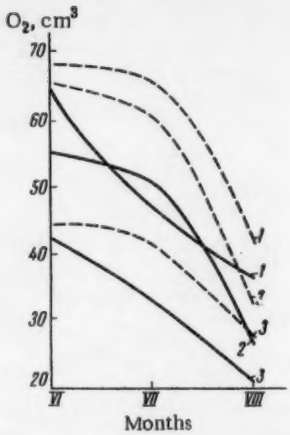

Fig. 2

Fig. 1. Height of the main cotton plant stem with and without irrigation.
1 and 2) With irrigation; 1a and 2a) without irrigation, 1 and 1a) variety K-922; 2 and 2a) variety 108-F.

Fig. 2. Catalase activity in cotton leaves in the dynamics of ripening. Solid lines – under irrigation; dotted lines – without irrigation.
1) variety S-460; 2) variety 108-F; 3) variety K-922.

The accumulation of soluble carbohydrates by cotton plants under insufficient soil moisture is considered to be due to inhibition of growth processes, as a result of which the flow of soluble carbohydrates from the leaves into other organs and parts of the plant is retarded. This position, expressed by Maksimov [3] becomes more obvious, since the photosynthesis in cotton plants decreases with insufficient water supply [4].

As indices of protein metabolism the content of total, protein, and nonprotein nitrogen was used. Soil drought increases the content of nitrogenous substances in leaves, including nonprotein nitrogen. The increase of the latter evidently is due to slowing down of protein synthesis as well as to partial hydrolysis. Hydrolysis of protein into more mobile forms makes it possible for nitrogenous substances to flow into reproductive organs. At the same time, despite the consumption of nonprotein nitrogenous substances by the fruit elements of the shrub, their quantity remains increased in the leaves in this case. The ratio of the nonprotein to protein nitrogen in the cotton leaves during the flowering period was: with irrigation 0.11-0.19, without irrigation 0.14-0.24.

The cotton plant is among those with high respiration intensity. It is also known that respiration indices of cotton plants change markedly in relation to water supply [4]. Accordingly, in the present study we gave attention to such biochemical indices as catalase and ascorbic acid which participate directly in oxidative processes, and particularly in plant respiration.

Under conditions of a nonirrigated background, the catalase activity was increased in all cotton plant specimens while varietal or species characteristics were preserved (Fig. 2). Toshchevikova et al [5] notes an increase of catalase and peroxidase activity in cotton leaves when irrigation is delayed.

In cultivating cotton plants without irrigation noticeable changes occur in the content of ascorbic acid with a tendency toward an increase, which is to a certain degree related to the delay of flow into other plant organs while growth processes are inhibited. And yet, this phenomenon is not without biological meaning. A high ascorbic acid content with a simultaneous increase of catalase activity may serve as an index of greater respiration intensity in nonirrigated cotton plants.

On a sector without irrigation the harvest of raw cotton was 30-45% of that on the irrigated sector. The limited assimilative surface of the cotton plant bush, as a result of retarded growth and decreased photosynthesis

[4] on the one hand, and the loss of substance on the respiration processes on the other, were the reasons for low cotton productivity under conditions of insufficient water supply.

TABLE

Dynamics of Oil, Protein, and Gossypol Accumulation in Cotton Seeds When Cultivated on Different Agricultural Background (variety 108-F)

Age of ball in days	Weight of 1000 seeds, g	Percent of kernels in seeds	In percent on dry basis: Protein (N x 5.5)					
			in kernel	in seeds	in kernel	in seeds	in kernel	in seeds
			Irrigated					
30	6.6	38.0	36.8	14.0	32.3	12.3	0.55	0.21
35	8.0	44.0	37.8	16.8	31.8	14.0	0.95	0.42
40	10.3	53.8	39.9	21.5	31.9	17.2	1.00	0.54
45	10.3	56.2	40.9	23.0	31.4	17.7	0.98	0.56
50	11.2	56.8	42.1	23.9	32.9	18.6	0.89	0.51
Opening of bolls (60)	11.8	57 7	41.8	24.1	33.2	20.2	0.87	0.50
			Nonirrigated					
30	7.5	51.7	38.1	20.7	34.4	17.8	0.69	0.36
35	7.7	53.5	39.2	21.0	33.2	17.8	0.69	0.37
40	9.1	52.5	41.1	21.6	33.9	17.8	0.74	0.39
45	9.4	55.7	40.0	22.3	34.7	19.3	0.71	0.41
Opening of bolls	9.5	56.4	39.7	22.4	35.6	20.0	0.69	0.39

Changes in cotton plant metabolism with differences in water supply are reflected in the tempo of accumulation of chemical substances and in their quantitative content in ripe seeds.

Above all, it should be noted that the period from flowering to the beginning of boll opening depends greatly on the presence of moisture in the soil. With irrigation, the period of boll ripening is longest in varieties 2 and 3 (over 70 days). In an average-ripening variety (108-F) it is 58-60 days; the fastest ripening species is an Indo-Chinese one – specimen K-922 (48 days).

On a sector without irrigation the duration of boll ripening is shortened considerably: in the Peruvian cotton plant (variety 2 and 3) by 18-20 days; in the Mexican (108-F) and Indo-Chinese (K-922) by 12-18 days.

With insufficient soil humidity the process of oil accumulation in seeds of an average-fibered cotton plant ends at a 40-day growth. However, with optimum soil humidity (65-70% of moisture capacity) the oil in the seeds is accumulated for a protracted period and its percentage by the time of complete ripeness attains (depending on species and variety in 50-70 day old seeds) a considerably higher figure than when cultivated without irrigation.

By the beginning of opening (cracking) of bolls, basic accumulation of substances in the seeds is ended. However, during the period from the beginning to the full boll opening, an increase of up to 1% oil as well as protein was observed. Undoubtedly, the presence of high moisture in the seeds the first days after boll opening is favorable for biochemical processes, particularly for oil formation.

From the data in the table it is evident that a considerable increase in the weight of seeds with irrigation is observed between the 35th and 40th day of boll development. A correspondingly marked increase in the percentage of kernels (by 8.8-9.8%) occurs at this period. Subsequently, the increase in the weight of seeds up to the end of ripening occurs at a more moderate tempo. An accelerated accumulation of oil, protein, and gossypol was also observed on irrigated sectors between the 30th and 40th day of boll development. Without irrigation the period of intense formation of these substances in the seeds occurs considerably earlier. Therefore, beginning with the 30th day of development, only a gradual and rather inconsiderable increase of substances in the seeds could be observed, and this ceased after 40 days of seed growth.

The accumulation tempo of substances in the seeds of studied cotton varieties under different backgrounds of cultivation is dissimilar. Thus, in seeds of 35 days growth in variety K-922 (G. arboreum) cultivated with irrigation, we found 91% oil and 75% gossypol compared to the content of these substances in ripe seeds (taken as 100%). In seeds of the same age in varieties 2 and 3 (G. barbadense), the oil and gossypol content were respectively 59% and 20% of their content in ripe seeds. In cultivation without irrigation the oil concentration in seeds of 35-day-old growth of variety K-922 comprised 96%, gossypol 98%; in varieties 2 and 3 these figures were 90 and 77%, respectively.

The tempo of seed ripening in the upland type under conditions of insufficient water supply is shown in the tabular data. If the maximum content of substances (in ripe seeds) is taken as 100%, the, when irrigated, the seeds of a 35-day growth of variety 108-F contain: 58% oil, 66% protein, and 41% gossypol. In seeds grown for the same period, cultivated without irrigation, there is 92% oil, 89% protein, and 93% gossypol.

Varietal differences in content of chemical substances in the seeds were manifested during the entire period of cotton plant ripening under different conditions of soil humidity. It should be noted that the percentage of oil as well as gossypol diminishes considerably under conditions of a nonirrigated background.

Data on dynamics of oil and gossypol accumulation in cotton seeds on different backgrounds of cultivation confirm the throught that the observed correlation between accumulation of oil and gossypol occurs as a result of similar action of external conditions on the independent processes of oil and gossypol formation [6].

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EFFECT OF PHOSPHORUS-POTASSIUM NUTRITION AND LIME ON RED CLOVER

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As is known, phosphorus, added as a fertilizer, is readily fixed by the soil and is transformed into a condition unavailable to plants. Complete prevention of precipitation of the phosphate of the fertilizer by the soil has not yet been found fully possible. Therefore it is expedient to introduce phosphorus fertilizer, taking into account the short duration of its action, at that period of plant development when it will give good economic value by considerably improving plant nutrition. In the belief that this aim could be attained by partial introduction of fertilizers, the effect of small doses of phosphorus-potassium nutrition and lime on survival of red clover under unfavorable fall and winter environments was investigated.

The experiments were conducted on sectors of the biological station of Kazan State University on small plots under conditions of 7-field crop rotation in 1951-1953 and 1956-1957. The plot size was 200 m^2. The experiments were carried out in triplicate. The soil was grey, weakly podzolic, of light mechanical consistency. The basic fertilizer in the form of peat in quantities of 20 ton/hectare was added to a fallow field before planting the cover crop, and was plowed in to a depth of 20 cm. In the control, clover mixed with timothy was cultivated without nutrient additions, on a background of the basic fertilizer. In the experimental variant the plants, in addition to peat, received phosphorus-potassium nutrition after the first clover mowing of the second year of growth (the first year of use). The nutrient was spread on the surface and dug in by harrowing, trailing it twice. The phosphorus nutrient was added in the form of superphosphate calculated as P_{30}, and the potassium nutrition was added as the potassium salt calculted as K_{30}. In 1951-1953 the liming of sectors of experimental variants was conducted on the basis of 1.5 tons/hectare ($^1/_2$ hydrolytic soil acidity). Soil pH (salts extraction) was 5.3. In 1956 on sectors where plants were cultivated, soil acidity was not high (pH 6.0), and therefore no lime was added.

In 1951-1952, 1956-1957 3 squares were established on each sector of experimental and control variants measuring 0.5 x 0.5 m for follow-up, on which observations were conducted of clover development during summer, fall and spring. By recording the known quantity of standard specimens, the number of plants which died was established and the percentage of clover drop-out was calculated for the given period. In 1953 the clover drop-out during the winter period was determined as follows: a sample of second-year clover was taken together with the soil (soil area 0.5 x 0.5 m, thickness of soil layer 40 cm, in duplicate) in January, after severe frosts, and was cultivated under laboratory conditions. This work was conducted by I.A. Tarchevskii.

The clover hay harvest was also recorded. Data are given in Table 1.

The data in Table 1 show that phosphorus-potassium nutrition and lime, added in small doses, decrease the clover drop-out and increase the hay harvest. The winter of 1956-1957 was relatively warm, there were no severe frosts, the snow cover was median (50-60 cm), sufficient for plant protection from harmful frost activity; therefore no clover drop-out was observed. The increase in hay harvest in 1951 was 15.7%, and in 1956, 15.8%.

What explains the increase of clover resistance to frosts as influenced by phosphorus-potassium nutrition?

To answer this question numerous investigations were conducted of the carbohydrate and nitrogen metabolism and utilization of phosphorus by the plants. Here we shall limit ourselves by stating only a portion of the material obtained by the above-mentioned physiological indices. For biochemical tests, samples of the clover

TABLE 1

Data Characteristic of Drop-Out and Yield of Red Clover Hay

Year of experiment	Summer-fall drop-out, in %		Winter drop-out, in %		Hay yield, in kg/m²	
	Control	PK nutrition	Control	PK nutriton	Control	PK nutrition
1951 – 1952	9.00	0.00	14.15	7.22	0.50*	0.58
1953	Not recorded		20.00	0.00	—	—
1956 – 1957	7.0	0.00	0.00	0.00	0.19**	0.22

* On basis of clover and timothy hay yield.

** Clover hay yield only.

TABLE 2

Data on Biochemical Tests of Wintering Red Clover Organs (in mg/g dry substance)

Tested substances	November		April	
	Control	PK nutrition	Control	PK nutrition
Sugar	182.20	190.20	62.9	87.30
Starch	6.57	8.30	14.80	15.59
Nonprotein nitrogen	10.65	12.71	4.42	9.25
Inorganic and acid-soluble organic phosphorus [4]	2.89 IX	3.99 IX	4.99 III	5.31 III
Phosphatide phosphorus [4]	1.95 IX	2.44 IX	1.69 III	2.00 III

wintering organs (root ÷ wintering buds) were taken at 2 periods: in the autumn (November) and spring (April). The preparation of clover-root samples for biochemical analysis was conducted by the commonly accepted method [1]. The sugar content was determined by the Bertrand method [1] after hydrolysis of nonreducing sugars with 2% hydrochloric acid. The starch content was determined by the Palogeimo [2] method. The nonprotein nitrogen [1] was determined from aqueous extracts obtained from clover roots (after protein precipitation). The inorganic and organic acid-soluble phosphorus compounds and phosphatides were determined colorimetrically [3]. The data obtained for all the above biochemical indices are given in Table 2.

From Table 2 it is seen that the quantity of osmotically active substances in wintering organs of fed plants in both tests was higher by comparison with those which received no nutrient.

Content of sugars, nonprotein nitrogen, and inorganic and acid-soluble organic phosphorus compounds is considerably decreased toward spring by comparison with autumn, in the clover roots of both variants; however, in the wintering organs of fed plants it decreases less than in the same organs of clover which had not been fed, which may be explained either by a smaller loss in supporting clover life during winter, or by greater hydrolysis of high-molecular weight biochemical compounds. A lesser use of plastic substances by the roots of fed plants in the winter indicates that their life processes proceeded less actively by comparison with control plants. This means that the experimental plants (nutrition by PK) entered a deeper dormancy than the control. As is known, plants which are more dormant have higher frost-resistance [5]. From this it can be deduced that a lesser drop-out of clover which received phosphorus-potassium nutrition is explained by its entering a deeper dormancy by comparison with control plants.

The higher rate of survival of fed clover under winter conditions by comparison with the control, and entry of experimental plants into deeper rest can be explained by greater accumulation of plastic substances. Osmotically active substances, while increasing the concentration of cell content, at the same time diminish the eutectic point of protoplast freezing and thus protect plasma colloids from freezing. Therefore the experimental plants with a high concentration of cellular juice have greater frost-resistance [5, 6] than the controls. On the other hand, the reason clover increases its frost-resistance under the effect of phosphorus-potassium nutrition is its greater accumulation of hydrophobic compounds in its wintering organs, particularly phosphatides. Phosphatides accumulated in plasmalemma protect cell contents from freezing and from harm by small ice crystals. When the content of hydrophobic substances (phosphatides) is high, the probability of forming small ice crystals and their harming the boundary plasma layers for the most part is smaller under conditions of low temperatures than when their content in plants is low.

The nitrogenous nutrition of clover is accomplished through the action of tubercle bacteria. Therefore we counted the total number of tubercles on roots of fed and nonfed plants. It was found that on the roots of experimental plants the total number of tubercles, as well as the number active, was larger (total – 498, active – 221) than on the roots of control plants (total – 249, active – 157). The increase in total and active tubercles on roots of experimental plants compared to the control indicates an improvement in the clover's nitrogenous nutrition by the effect of phosphorus-potassium feeding and lime.

The above data indicate that feeding of clover by small doses of phosphorus-potassium fertilizer, as well as small doses of lime, improves nutrition; increases plant survival and hay harvest, which may indicate greater accumulation of plastic substances in wintering organs of fed plants; gives a small drop-out of clover, and a larger harvest by comparison with nonfed plants.

Based on the above, industrial tests are recommended on grey, weakly podzolic soils of light mechanical consistency of the Tatar ASSR as follows: phosphorus-potassium nutrition after the first mowing of second - year clover (the first year of use) by $P_{90}K_{90}$, with a simultaneous addition of lime at the rate of 1.5 tons / hectare ($\frac{1}{2}$ hydrolytic soil acidity), in order to increase its winter-resistance and yield.

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EFFECT OF TEMPERATURE VARIATION ON TOMATO SEEDS

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The question of the nature of effects of lowered or variable temperature has met with different reactions in the studies of many authors. Veronova [1] calls this method hardening. Filatov [2] sees the cause as stimulant formation at lower temperatures. This point of view was confirmed and given an experimental basis by Blagoveshchenskii [3]. A number of authors [4-7] established that the effect of lowered temperatures on seeds, as well as treatment by biogenic stimulants, increase the activity of a number of enzymes, especially protease. Recently, even the chemical nature of biogenic stimulants was established.

In addition to a purely biochemical study of the effects of lowered and varying temperatures, a diminution of the protoplasm viscosity is noted in hardened plants [8] which, the authors suggest, increases the cold-resistance of hardened plants and secures more intense growth during early phases of development on cold days.

In the studies [1, 9-11] it is shown that a single cooling of seeds is always worse than the effect of varying temperatures. In attempting this study our aim was to clarify the reason for the more favorable effect of varying temperatures.

METHODS

All the seeds were soaked for 24 hours at room temperature, then divided into 2 portions and placed in gauze bags. One portion was refrigerated at 0° for 12 hours during the day. The other portion was placed in a constant temperature room at +20° during the day. The seeds had a constant access to moisture. At night the seeds were switched for 12 hours. This treatment continued for 15 days. The control seeds were soaked 2 days before planting and were held at a temperature of +20°. The choice of the cooling temperature was made on the basis of a detailed study by K.D. Shchupak and N.N. Zaginailo. According to these authors' data, reduction of temperature below 0° does not allways give a positive effect.

Seeds from tomato varieties Mayak, Brekodei, Stalingradets, Chudo rynka (Market wonder), and Bizon were taken for the study. The treated and control seeds were planted in boxes on March 20, and then by the middle of April were transferred to seedbeds, in duplicate. The sprouts from the seedbeds were transplanted on May 17 to field single-stripped plots of an area 9 m^2, in triplicate. A portion of the sprouts was used to measure respiration rate during day and night exposure. Respiration rate was determined by the usual barytes method, in duplicate. In order to minimize errors, titration and equipping of vessels was conducted in the open air.

RESULTS

Differences in variants were found at the earliest period. The sprouts differed greatly in their growth processes. Variant No. 1 (control) fell behind the other variants. Variant No. 2 (seeds treated by cold by day and warmth at night) occupied an intermediate position. Variant No. 3 (seeds treated by warmth by day and cold at night) led the other variants. Plants of variant No. 3 were several times larger in April than plants of other variants. Subsequently the differences leveled out (Table 1).

Phenological observations of the flowering time also showed advantages for variant No. 3, which, of all varieties, began to flower 5-7 days earlier than the control, and 3-5 days earlier than variant No. 2. As an example the phenological observations of tomato variety Mayak are cited (Table 2).

TABLE 1

Height of Plants by May 15 (in cm)

Variety	Variant		
	1	2	3
Bizon	8.0	13.3	15.5
Mayak	16.5	12.6	22.2
Stalingradets	7.6	13.6	17.5
Brekodei	10.0	13.4	15.7
Chudo rynka	15.1	16.4	21.6

TABLE 2

Flowering of First (I) and Second (II) Tomato Clusters, Mayak Variety

Variant no.	I		II	
	Beginning	Full	Beginning	Full
1	6.VI	10.VI	11.VI	16.VI
2	5.VI	10.VI	12.VI	15.VI
3	31.V	7.VI	10.VI	13.VI

Subsequently considerable differences were observed between variants in crop yield, especially during early periods. The yield at an early period was highest in the third variant for all varieties except Stalingradets, in which the total yield was only slightly higher than in the others. In Table 3 the crop yield data are given for varieties Mayak and Brekodei 125.

TABLE 3

Tomato Yield of Mayak and Brekodei Varieties

Variant no.	Mayak yield						Brekodei 125 yield					
	July 28		August 4		August 18		August 4		August 16		Total	
	ctn/ha	%	ctn/ha	%	ctn/ha	%	ctn/ha	%	ctn/ha	%	ctn/ha	%
1	100	100	158	100	412	100	41.9	100	256.7	100	565.3	100
2	112	112	155	98	415	100.07	92.1	220	344.4	134	569.5	100.8
3	156	156	222	141	456	111.2	127.5	303	365.4	142	594.7	105.0

As indicated in Table 3, differences between variant No. 3 and the control are diminished with time, but the early crop is considerably higher than the control. Behavior of second variant plants of all varieties in the experiment differes. The total yield of variant No. 3 is higher for all varieties except Bizon.

On April 19 and 20 on the above-ground portion of seedlings varieties Mayak and Bizon respiration was measured upon day and night exposure. Results of the measurements were calculated per g raw weight. The data obtained (in mg CO_2/hour) for Mayak variety were as follows:

Seeds cooled	Respiration during day	Respiration at night	Average daily respiration
During day	0.3	0.151	0.225
During night	0.122	0.196	0.173

As indicated by the experimental data, during daytime hours the respiration is 2.45 times greater in variant No.2, the seeds of which received cold treatment during the day. During night hours the respiration of variant No. 3 at 20° is 1.2 times higher than in variant No. 2. The average daily respiration in variant No. 2 is higher than in variant No. 3 by 1.3 times. Since early spring temperatures in seedbeds at night are low (10-12°), the average daily plant respiration was determined mainly by the course of respiration during the day.

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DIURNAL DYNAMICS OF CHLOROPHYLL CONTENT IN PLANT LEAVES

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There are contradictory data in the literature on the question of quantitative pigment changes in plant leaves in the course of 24 hours.

A number of authors share the point of view expressed by Willstätter and Stoll [1] that the quantity of chlorophyll in plant leaves does not change over a short period. Thus Seybold and Egle [2, 3, 4] have shown that the chlorophyll content at different times of the day remains constant. Having found that there were no variations in chlorophyll during the day, Bauer [5] concludes that the view of chlorophyll stability is correct.

On the other hand, there are data which show that the chlorophyll quantity does vary. Bukatsch [6] and Wendel [7] have established that in natural environments the chlorophyll content of Alpine plants changes considerably. According to Bukatsch' data, these variations may reach 200-300%. In studies by Sironval (cited by Bauer [5]) variations in chlorophyll content during the day were found in young leaves of wild strawberries. Gyubbenet and Bazhanova [9], in studying the diurnal dynamics of chlorophyll in potato leaves, found that the chlorophyll content changed constantly, increasing during the daytime hours and decreasing toward 5-7 A.M. In individual experiments maximum chlorophyll was observed during morning hours. Variations in chlorophyll reached 25%.

Data of Turchin [10], Shlyk, Godnev et al [11, 12] obtained by exact methods using N^{15} and C^{14} showed that chlorophyll in plant leaves is continuously regenerated.

In view of contradictory data in the literature, during the summer of 1957, under the leadership of Professor M. Kh. Chailakhyan, experiments were conducted in the laboratory of growth and development in the K.A. Timiryazev Institute of Plant Physiology, AN SSSR, on the study of diurnal chlorophyll dynamics in leaves of short-day plants – red perilla and Japanese millet, and a long-day plant – rudbeckia.

Perilla and rudbeckia, planted on March 4-12, were cultivated on soil in vases; millet, planted May 25, in sand on a Knop nutrient medium in glass vessels.

Analysis of chlorophyll content was conducted on adult plants which were subjected to a long (natural) day and a short (10 hours) one, created by holding the experimental plants in a photoperiodic chamber. Samples taken from plants placed in darkness were analyzed in a dark room. Weighed portions from 2-3 healthy, physiologically active, and fully grown leaves were taken for analysis; in each variant the weighed portions were taken from 3-4 plants. Determination of chlorophyll content was conducted by the Godnev method using a Kening-Martens spectrophotometer. The regularity in the diurnal chlorophyll dynamics was alike when converted per unit of raw and dry weight, as well as in conversions per unit area. Therefore in Figures 1, 2 and 3 data are given only by calculating the chlorophyll content per unit area.

The following data were obtained from the experiments. One or two maxima in chlorophyll content were observed in light in the plants held on a long day as well as in the plants held on a short day. Variations during the day were in the range of 10-40% on as-is basis, 25% on dry basis, 15-25% on unit area basis. At night the chlorophyll content of plants held on a long day did not change. The chlorophyll content of plants held on a short day was changed during a long night. This was shown by the fact that the original, sometimes considerable, loss of chlorophyll was changed to a considerable increase. Variations in darkness attained 10-20% on as-is basis, 10-30% on dry basis, and 10-20% per unit area. Thus, our data show that the chlorophyll content changes not only in light but in darkness.

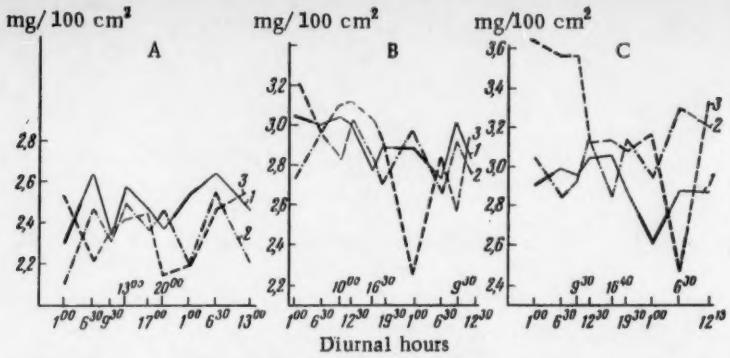

Fig. 1. Diurnal dynamics of chlorophyll content in red perilla leaves (in mg/100 cm²).
A) July 1-July 2: 1) plants held on a long day throught; 2) plants held on 3 short days; 3) plants held on 17 short days. B) July 8: 1) plants held on a long day throughout; 2) plants held on 10 short days; 3) plants held on 24 short days. C) July 15-July 16: 1) plants held on a long day throughout; 2) plants held on 17 short days; 3) plants held on 31 short days. Thick line–night; thin line–day.

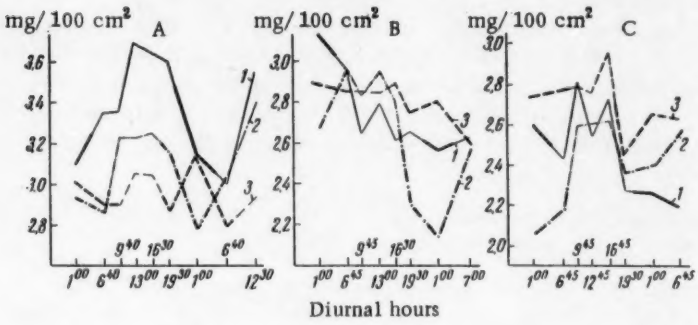

Fig. 2. Diurnal dynamics of chlorophyll content in leaves of Japanese millet.
A) July 30-July 31: 1) plants held on a long day throughout; 2) plants held on 3 short days; 3) plants held on 18 short days. B) August 6-August 7 1) plants held on a long day throught; 2) plants held on 11 short days; 3) plants held on 25 short days. C) August 13-August 14: 1) plants held on a long day throughout; 2) plants held on 18 short days; 3) plants held on 32 short days. Thick line–night; thin line – day.

Changes of chlorophyll content effected by light and darkness in sprouts depend, according to Frank's [13] data, on the following processes: 1) chlorophyll synthesis in light, 2) decomposition of chlorophyll in light, and 3) decomposition of chlorphyll in darkness. In addition to this scheme, we succeeded in finding a variant in which the chlorophyll quantity increased in darkness in grown plants placed on a 10-hour short day. The increase in chlorophyll content in darkness was also found in experiments of Vlasenko and Dombrovskaya [14], in which citrus plants were subjected to the effect of prolonged darkness.

It is known from the literature that, of the higher plants, the conifera [15, 16] can synthesize chlorophyll in the dark. A number of authors express the hypothesis that there is an enzyme system in plastids of conifera producing a reduction of protochlorophyll independent of light. Sprouts of higher plants acquire the capacity of chlorophyll synthesis in darkness when enzyme systems of conifera are added to etiolated leaves, as was done by Godnev [16].

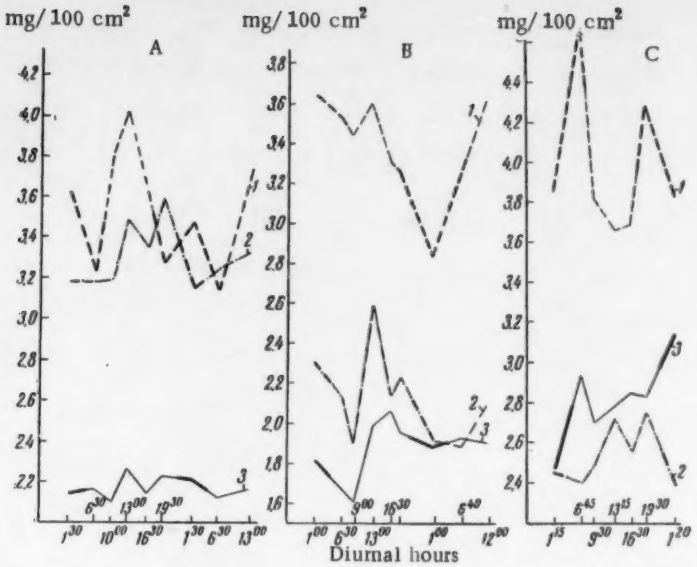

Fig. 3. Diurnal dynamics of chlorophyll content in rudbeckia leaves. A) July 18-July 19. 1) plants held on a short day throughout; 2) plants held on three long days; 3) plants held on 17 long days: B) July 25-July 26: 1) plants held on a short day throughout; 2) plants held on 10 long days; 3) plants held on 24 long days. C) August 3-August 4: 1) plants held on a short day throughout; 2) plants held on 19 long days; 3) plants held on 33 long days. Heavy line – night; thin line – day.

The fact that the amount of chlorophyll increases in darkness, as found by us, evidently can be explained on the basis that in grown flowering plants, as well as in conifera, there is one enzyme system or another which participates in chlorophyll synthesis.

Apparently chlorophyll synthesis in flowering plants can be accomplished both due to light energy and also in darkness from respiration energy.

Consequently, the chlorophyll content of leaves of grown plants is not constant during the day; changes in chlorophyll content are conditioned by these processes: a) chlorophyll synthesis in light, b) decomposition in light, c) decomposition in darkness, and d) by synthesis in darkness.

The author expresses hisgratitude to the scientific director, Professor M. Kh. Chailakhyan, and the chief scientific collaborator O.P. Osipov, for aid in this study.

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** See English translation.

VARIETY OF PHLOX PLANTS OBTAINED FROM ROOT CUTTINGS OF VARIOUS AGES

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We know that the vegetative progeny carry, as a rule, the imprint of development both of the entire organism and of the separate organ [1-3]. The applicability of the indicated position to the vegetatively derived progeny of root and rhizome origin still is inadequately studied.

The idea is expressed that the natural regeneration of plants by root suckers leads to the rejuvenation of the race [4,5]. Kazaryan [6] describes the differences in the fermentative activity and the dates of sprouting of the first fruit stems for progeny obtained from roots from different places within the limits of the root system of the plant. However, the data of V.O. Kazaryan do not give an adequate presentation of the ontogeny of vegetative progeny in view of the absence in the work of an indication of the tempo of their development in the following years.

In our work we studied the periods of budding and flowering for progeny from roots of various ages during a period of two years. At the same time the progeny were also studied in relation to the regenerative ability of leaf cuttings.

The work was carried out in 1955-1956 in the Botanical Garden of Leningrad University under the direction of K.M. Zavadskii, to whom we give our deep thanks.

METHOD

The varieties of snow phlox (Phlox paniculata L.) Panama and Joan of Arc were used for the experiments.

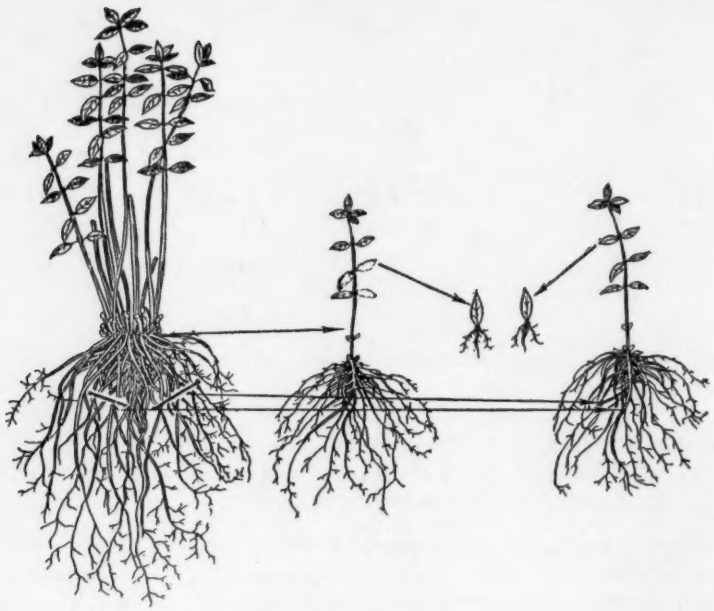
The vegetatively derived progeny obtained from young and old portions of the root system of phlox were studied (see Fig.).

Phlox bushes that had overwintered in the open ground were dug up at the end of April and young and old roots with buds were isolated from their root systems. Cuttings from the old roots were taken from the lignified zone of the root collars, which is the oldest part of the bush. The young roots were the thick lateral roots, formed to the degree of the growth of the root collar [7].

The cuttings from the old part of the root system used in the experiments were twice as large as the cuttings from the young roots. The cuttings of young and old roots were grown in individual jars which were placed on shelves of a greenhouse with the experimental material and watered regularly. Shoots with eight to nine small leaves appeared from the buds of the isolated roots toward the end of May. The first generation progeny of the young and old roots were placed in the ground in the first part of June where they grew under natural conditions.

The first generation leaves of the upper stage were used as cuttings. The isolated leaves were grown in jars with sand and rooted in the greenhouse with a high moisture substrate and air temperature of 23-27° in conditions of natural light. The intensity of root formation and the growth of the root system for cuttings were observed regularly.

The leaves of comparable vegetatively derived progeny were suckered in three periods: July 7, 1955, June 5, 1956, and July 1, 1956. The results obtained in all periods of suckering were similar.



Scheme of obtaining vegetatively derived progeny from the root system. Left, parent plant; middle, plants from old roots; right, plants from young roots.

RESULTS OF THE EXPERIMENTS

We see from Table 1 that the vegetatively derived progeny from the old portion of the root system in the first year of life flowered earlier than the progeny from the young roots, in spite of the absence of significant differences in growth.

TABLE 1

Growth and Development of Phlox Plants (Panama) Obtained from Root Cutting of Various Ages (1956 experiment, first year of life)

Progeny from roots	Number of plants	Vegetative condition of single plant on August 5			Percent budding on August 5	Percent flowering	
		Height of stem, in cm	Number of leaves per shoot	Dimensions of leaf, in cm*		August 5	September 9
Old	120	54.8	33	9.1 x 3.0	58.3	5.0	85.1
Young	35	47.2	31	10.1 x 3.2	2.8	2.8	14.4

* Dimensions of leaf reflect average data for leaves of the third level from the point of growth.

In the following years the progeny of the root cuttings of various ages did not differ in the date of flowering. Thus, in the second year of the experiment, that is, after the experimental plants had overwintered in the ground, differences in time of flowering were not observed for progeny of the young and old roots. Similar results were obtained for the Joan of Arc variety.

TABLE 2

Root Formation of Leaf Cuttings of Phlox (Panama variety) from Plants from Roots of Various Ages (1955 data, first year of life)

Progeny from roots	Number of cuttings	Percent of rooted cuttings by days					Vigor of root system of one cutting	
		36th	42nd	54th	60th	68th	Number of rootlets	Length, in mm
Old	53	3.7	6	26.6	32.0	52.8	1.4	47.5
Young	23	8.7	17.4	33.0	46.2	65.2	1.6	55.6

TABLE 3

Root Formation From Leaf Cuttings of Phlox (variety Panama) From Plants Obtained in the Preceding Year From Root Cuttings of Various Ages (1956 data)

Progeny from roots	Number of cuttings	Percent of rooted cuttings by day of observation				Average vigor of the root system of one cutting	
		20th	30th	50th	60th	Number of rootlets	Length, in mm
Old	127	14.8	43.2	69.1	77.1	1.9	57.6
Young	85	36.4	75.3	87.0	88.2	2.8	63.4

We see from Table 2 that the leaf cuttings of plants from young roots rooted better and developed a more vigorous root system than the leaf cuttings of plants from the old part of the root. These differences were maintained in the second year of their lives (Tables 2 and 3).

Our experiments in relation to the data in the literature [6] show that the date of flowering of vegetatively derived progeny depends on the place from which the root cuttings were taken within the root system of the plant. Plants obtained from the root collar proceeded to budding and flowering significantly earlier than plants from the young lateral roots.

The variety of vegetatively derived progeny depends mainly on the characteristics of the suckering of the root. Probably the root collar of phlox is distinguished from the young lateral roots not only by age, but also by staged preparation, inasmuch as it is formed directly from the tissues of staged old shoots. The qualitative differences of the root cuttings, for which their retention of a connection with the formed sprouts in ontogenesis [8] must be considered a basic reason, are an instrumental manifestation of the variety in progeny from root cuttings of different ages.

The differences in the regenerative ability of leaf cuttings for progeny from roots of various ages are more stable than the differences in the time of flowering. In the first case, these differences are firmly retained even after the disappearance of the differences in the time of flowering in their second year of life. Obviously, the differences survive for vegetatively derived progeny from roots of various ages in the second year of life (that is, on the concurrence of their flowering), in particular, the age differences which result in the dissimilar rooting of leaf cuttings.

Our data do not agree with the idea of Pilipenko [4] and Yurtsev [5], according to which a complete rejuvenation takes place for additional buds formed in the root system. Our experiments show the variety of additional shoots formed in the root system of the plant.

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SOME BIOCHEMICAL PROCESSES FOR GRAPE VARIETIES DIFFERING IN FROST RESISTANCE

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It is obvious from the large number of investigations appearing in the literature that frost resistance and other biological features of the plant are closely connected with the characteristics of the metabolism of substances. Consequently, one of the methods for the study of frost resistance of plants can be the comparative investigation of the individual links of the metabolism of substances for plants with differing frost resistance.

The results of the study of several biochemical features of the grape vine in connection with its frost resistance are given in the indicated communications. The frost-resistant Michurin and nonfrost-resistant domestic varieties of grape of approximately the same age and date of ripening and grown under similar soil-climatic conditions served as the objects of the investigation. Northern White and Russian Concord were among the frost-resistant varieties and Seedling Malengra was used as the relatively poorly frost-resistant variety; the nonfrost-resistant varieties were Spitak Arakseni, Spitak Sateni, and Sev Arakseni.

The leaves in the period of formation and the growth of the grapes and shoots for the entire annual cycle of the vine were subjected to analysis. The first tests for the analysis were made using the green shoots during the period of flowering and after flowering, following this in relation to the lignification of the shoots, and then in the autumn-winter period and the spring of the following year after digging up the grape vines.

The activity of the catalase in the leaves was determined by the iodometric method, the concentration of ascrobic acid by the Prokoshev method (see Belozerskii and Proskuryakov [1]) and the degree of the ordinary oxidizability of the leaves using a 0.1 N solution of potassium permanganate which can, to a certain degree, give an indication of the concentration of oxidizing substances in the given object.

The quantity of starch in the samples of the shoots fixed with flowing vapor was determined by the combination method with the action of the enzyme of diastase and acid hydrolysis with 2% HCl.

The data obtained on the activity of the catalase of the leaves shows (Table 1) that the activity of the catalase for frost-resistant varieties is significantly higher than that of nonfrost-resistant varieties both at the beginning and at the end of the growth of the fruit.

We previously observed [2] the higher activity of the enzyme of peroxide in the leaves of frost-resistant varieties. Thus the data obtained can bear witness to the high level of the oxidizing processes for frost-resistant varieties of grape.

According to the data of Grebinskii [3], the sharp increase in the concentration of ascorbic acid and the activity of the catalase and peroxide are observed in conditions favorable to the development of frost resistance in plants.

The data on the accumulation and conversion of starch in the shoots for varieties with different frost resistance shows (Table 3) that the growth and lignification of the shoots is accompanied by the accumulation of starch in them; however the tempo of its accumulation is not the same for various varieties of grape according to their frost resistance.

TABLE 1

The Activity of Catalase in the Leaves of Grape Vines, in ml 0.1 N H_2O_2 per 3 Minutes per 1 g of Dry Substance

Variety of grape	Beginning of growth of the fruit. June 21	End of growth of the fruit. July 13
Frost-resistant		
Northern White	16.70	13.80
Russian Concord	13.70	13.90
Seedling Malengra	—	16.20
Nonfrost-resistant		
Spitak Sateni	4.10	3.78
Spitak Arakseni	1.00	5.83
Sev Arakseni	0.74	1.53

TABLE 2

Concentration of Ascorbic Acid and Ordinary Oxidation in the Leaves of Grape Vines.

Variety of grape	Ascorbic acid, in mg % of dry weight		Ordinary oxidizability 0.1 N $KMnO_4$ per 1 of dry weight
	Beginning of growth of fruit	End of growth of fruit	
Frost-resistant			
Northern White	1131	502	5.00
Russian Concord	799	452	6.40
Seedling Malengra	1098	365	5.25
Nonfrost-resistant			
Spitak Sateni	581	304	4.25
Spitak Arakseni	612	301	4.25
Sev Arakseni	642	307	4.00

TABLE 3

The Accumulation and Conversion of Starch in the Shoots (in % of dry weight in conversion to glucose)

Variety of grape	Vegetative period					Autumn-winter period		
	31.V	26.VI	18.VII	5.VIII	19.IX	19.XI	2.II	25.III
Frost-resistant								
Northern White	2.75	4.63	6.75	6.90	11.70	5.89	3.33	6.41
Russian Concord	2.45	2.32	4.68	7.95	12.17	8.58	5.71	9.83
Seedling Malengra	2.92	2.05	5.58	7.19	13.50	14.04	6.95	11.79
Nonfrost-resistant								
Spitak	2.25	2.31	2.91	5.32	9.83	10.88	7.85	9.33
Spitak Arakseni	1.70	1.72	2.91	4.57	11.50	12.70	7.47	10.73
Sev Arakseni	2.03	2.36	2.39	4.64	9.51	11.80	7.34	12.44

The frost-resistant varieties have a reserve of starch in the lignified shoots beginning during the earlier stages of lignification (June-July) and proceeding more intensively than for the nonfrost- resistant varieties (at a similar degree of maturity of the sprouts).

The indicated shift toward the accumulation of starch in the shoots of nonfrost- resistant varieties was noticed only at the beginning of August when the growth processes in the vine are weakened. However, the high level of concentration of starch in frost-resistant varieties is maintained throughout its entire vegetative period, as a result of which the autumn starch maximum is reached earlier than in nonfrost-resistant varieties.

It is known that, in addition to the reserves of starch, its further conversion to sugars in the autumn-winter period is also important for safe wintering of plants.

According to our data the conversion of starch in the shoots does not proceed in like manner for varieties with different levels of frost resistance.

We can see from the data in Table 3 that the quantity of starch in the frost-resistant varieties dropped sharply after the first frost (Nov.19), while at the same time there was practically no change in the nonfrost - resistant varieties. The decomposition of starch in the latter began only after a significant lowering of the air temperature. The starch minimum in all varieties was recorded in February, coincident with the lowest temperature. However hydrolysis for frost-resistant varieites and, in particular, for the variety Northern White proceeded to such a degree that the remaining starch in the shoots was only half as much as for the nonfrost- resistant varieties.

Sergeev and Sergeeva [4] noted in their investigations that the tissues of plants containing starch granules in the winter exhibit an increased susceptibility to low temperatures. In spite of the substantial explanations and hypotheses, the question of the importance of the conversion of starch for the frost resistance of plants still remains unclear.

We can see from Table 3 that there is a resynthesis of starch after the winter period with an increased air temperatures, as a result of which the quantity of starch in the shoots increases. A second starch maximum (spring) is reached in the annual cycle of the vine. The vine prepares itself by this for vegetation.

Thus frost-resistant varieties of grape are distinguished from the nonfrost - resistant varieties in the period of formation and growth of the grapes by the increased activity of catalase, the higher concentration in the leaves of ascorbic acid and the high degree of ordinary oxidizability of the leaves, shown by the higher level of the oxidizing processes for frost-resistant varieties. The accumulation of starch in the shoots of the frost-resistant varieties in the period of vegetation begins somewhat earlier and proceeds more intensively than in the nonfrost - resistant varieties, as a result of which the frost-resistant varieties are characterized by a higher concentration of starch in the shoots. An earlier and more complete decomposition of starch is observed in the autumn-winter period for the frost-resistant varieties.

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DESCRIBING THE FORM OF FRUITS, LEAVES AND OTHER ORGANS OF PLANTS

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It is very important to study the form and relief of fruits in the search of a means to improve and uncover large varieties. The larger the percent of large fruits given by one or another variety of fruit, vegetable or other crop, the higher is its commercial value, and vice versa.

The size, weight, color and form of the fruit constitute the characteristic biological basis of the variety in spite of the significant variability of nutrition.

The dimensions of fruits usually are measured by the height and diameter of the fruit, using its largest diameter. A sliding calipers is used for such measurements. However such measurements do not always give true indications because the size of the fruit does not depend only on its volume. Cases are often encountered where the fruit with the largest dimensions has the lowest weight, and vice versa. It is clear that it is possible to characterize the size of the fruit only when measuring these two factors (volume and weight).

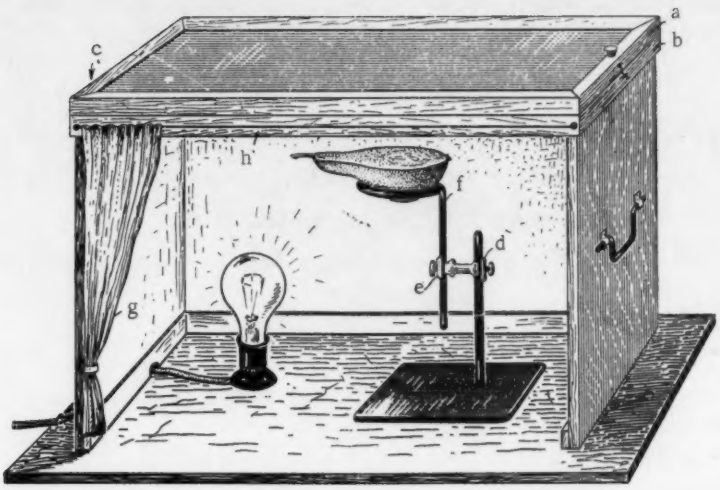
Even long ago many researchers studied the nomenclature of the form of fruits. Thus, German pomologists established a specific terminology for the various forms of fruits. For example, they gave twelve forms for apples: flat, half-round, flat-round, spherical, oval, oval-round, cylindrical and others [1].

Even in the 1880's [2,3] attempts were made to measure the size of fruits, and also to study the characteristics of the contours of externalconfigurations of fruits both of typical forms and of every deviation from the standard type of the given variety. The substance of the investigation of M.V. Rytov lay in the fact that he attempted with the aid of contours of cross sections and curves to study and perceive the rules of growth of fruit and various deviations under the effect of a variety of reasons. Novikov-Golovatyi [4] used the method of mathematical ex - pression as the criterion giving the most objective determination to determine the form of apples and pears. For this, he studied with the aid of a glass millimeter grid the size of different lines characterizing the separate elements of the configuration (height, diameter of the fruit, depth of cupped depressions, height of the core, etc.).

In contrast to earlier investigations, ours took into account the perimeter (longitudinal and transverse) for the study of the size of fruit. We developed special graphs, using the statistical variation for calculation of their size, which show the volume of the fruit in relation to the ratio of the longitudinal size to the transverse [5].

The authors of the methods indicated above of characterizing and measuring the form and size of fruits and also of many works having a direct connection with the given question did not indicate the method with which they studied the morphological features of fruits. The question on the methods of describing the sections of the fruit is especially important. This is important because the presence of simplified means of describing the form of longitudinal and transverse sections would ease somewhat the analytical investigation of vegetable and fruit crops and their change in relation to the idea of developing a simple and exact means of describing the configuration of fruits.

Usually the researchers proceed in the following manner when describing the configurations of fruits: the fruit is cut in the longitudinal and transverse directions and the cut side is placed on paper after which its contour is traced with a pencil. The core of the fruit (seed box, axial cavity, tube, etc.) is also outlined with a chemical pencil in order to obtain its contour, and a tracing is obtained when placed on paper. Often the configuration of the fruit is outlined with a chemical pencil or ink at the same time for both the border and for the internal picture of the fruit.



The instrument for describing the form of fruits, leaves and other organs of plants.

The prints obtained by this means do not give the exact outlines of the fruit (usually one gets a wide line and the ink spreads along the pulp of the fruit). Apart from this, the method does not work for describing the form of juicy fruits: tomato, several varieties of pear, watermelons, cucumbers, muskmelons, and others.

The description of the configuration of fruits that we did was carried out with the help of a special instrument (see Figure).

The instrument consists of a wooden box, to the top of which are riveted two frames with glass, a and b. The frames are connected on one side with hinges, c. A stand with a clamp, d, is placed inside the box; the other end of the clamp, e, holds a ring, f, designed for holding the fruit is on top of it. A stationary stand with an electric light is on the base of the box (on the left side). The front part of the instrument is a pull curtain of black material g, which slides on the fine wire, h.

The description of the longitudinal and transverse sections of the fruits is carried out with the help of the instrument described above in the following manner: the fruit is moved up against the glass with the help of the stand prior to starting to outline the form of the fruit, and outlined. Only the lower frame must be used for drawing. In this case the upper frame is opened and all parts of the fruit are outlined with India ink on the glass of the lower frame. After this the electricity is turned on and then a Whatman or gelatin paper is placed on the glass (with the outlined contour of the fruit) and copied. Description by our method gives a clear representation of the natural form of the fruit. Besides this, a basic goal in our method is the possibility of calculating the interior of the fruit, for example, the chamber of the tomato, the place where seeds are located in juicy fruits, the veins of leaves and flowers, and others.

The configuration of the fruit can also be transferred on tracing paper. In this case it is not necessary to turn on the light. The outline of leaves, flowers and other organs of the plant can be traced with the help of the given instrument. For this the leaf or flower must be placed on the glass of the lower frame and the upper frame closed. If it is necessary to photograph the leaves or flowers, the light is turned on and the impression is transferred to paper.

Observations show that the size of the surface of leaves can also be measured when describing their form. Good results are given by our method of measuring the size of the surface of leaves [6].

There is no necessity to draw on the glass with India ink when tracing the contour of the leaves using the indicated instrument. This can be done with the help of the lighted lamp, but if tracing paper is used in place of the Whatman paper there is no need to use the light.

One must remember when building this instrument that the space between the glass of the upper and lower frames does not exceed 1-2 mm. This is necessary in order to straighten the study objects. The size of the box has to be coordinated with the dimensions of the fruit.

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DYNAMICS OF PLASTIC SUBSTANCES AND WINTER-HARDENING OF CLOVER IN RELATION TO PHOSPHORUS-POTASSIUM NUTRITION CONDITIONS

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The mortality of grasses during the wintering period results in great damage to Socialist agriculture and livestock. For this reason the development of means to increase the winter-hardening of clover is a primary problem for agrobiological science and agricultural production.

The correct application of fertilizer is of great importance in increasing the winter-hardening of clover. The question of the effect of mineral fertilizers on the winter-hardening of clover under various soil-climate conditions is still inadequately studied, in spite of a number of achievements in this area. The effect of nutrition conditions on the change of metabolism of substances in the winter organs of clover has been studied only slightly.

In connection with this, our problem was the study of the effect of phosphorus and potassium fertilizer on winter-hardening of clover in the conditions of the L'vov region. In this region, as in other westerly regions of the Ukraine, the winter weather is characteristically variable. This is an area of thaws and sharply fluctuating temperatures, where a frozen crust is formed. Clover may die in this region during the winter from the abrupt changes in temperature in the absence of a snow cover, which disappears as a result of the frequent thaws, from freezing, from the frozen crust, and to a lesser extent, from thawing, absorption of water, and frost heave. These or other unfavorable conditions develop to a greater or lesser degree, depending on the character of the winter. The alternate thawing and freezing generally after the arrival of snow, can induce a higher percent of mortality of winter-killed plants. In some of the more unfavorable years, the mortality ofclover can reach a significant level.

The winters in which our experiments were conducted, 1949-1950 and 1951-1952, were characterized by unfavorable conditions for overwintering, although the winter of 1950-1951 was more favorable. The mortality of clover during the winter of 1949-1950 generally resulted from freezing and occasionally from the frozen crust. In the winter of 1951-1952, clover suffered to a great degree from the unfavorable conditions in the winter-spring period and mortality in plants was caused by freezing, frozen crust, and occasionally by thawing and being wetted.

We studied the effect of nutrition conditions on the death of plants, and also on the concentration of plastic substances in the winter organs during the first two years of the life of clover. The experiments were established on the experimental section of the L'vov Pedagogical Institute (winters of 1949-1950 and 1950-1951) and on the Shevchenko collective farm (winters of 1950-1951 and 1951-1952) with local types of clover. The clover was grown under field conditions on gray-forest medium-clay soils with the pH of the salts ranging between 6.1 and 6.4.

The phosphorous (superphosphate) and potassium (potassium salt) fertilizers were used in the form of food in the autumn for first-year plants, and in early spring and after the first mowing for a second-year plants. At every application the fertilizers were spread on the surface in a mixture at 30 kg per hectare of active substances for each type of fertilizer. The mortality in clover was studied on especially laid out permanent plots on which the number of living plants of clover was determined at the beginning of winter and in the spring after overwintering.

TABLE 1

The Effect of Fertilizer on the Overwintering of Clover in the First and Second Year of Its Life.9 (percent of living plants after overwintering)

Experimental variant	Experiments on L'vov Pedagogical Institute		Experiments on Shevchenko collective farm	
	First-year clover	Second-year clover	First year clover	Second-year clover
Control (not fertilized)	54.6	81.7	20.1	93.6
P	28.7	79.2	12.5	92.4
K	36.4	70.1	15.3	80.8
PK	19.8	60.4	6.7	76.3

TABLE 2

The Change in the Concentration of the Sum of Sugars in the Root Collars of Clover at Various Times of Overwintering (as percent of dry weight)

Experimental variant	First-year clover			Second-year clover		
	Beginning of winter	Middle of winter	End of overwintering	Beginning of winter	Middle of winter	End of winter
Experiment section of L'vov Pedagogical Institue						
Control (no fetilizer)	16.21	15.82	9.07	10.37	9.82	6.83
P	19.14	20.08	12.25	10.81	9.09	7.54
K	18.52	18.26	11.42	13.26	12.54	8.08
PK	21.35	22.63	14.24	15.45	15.17	9.43
Experiment at Shevchenko collective farm						
Control (no fertilizer)	17.16	16.41	9.63	11.43	10.62	5.27
P	20.09	19.24	11.91	12.25	10.36	4.82
K	19.36	20.12	10.47	12.71	12.58	6.45
PK	23.53	23.05	12.82	16.43	15.91	8.33

In the winter organs of clover (root collars) the concentration of the sum of the sugars was determined according to the Bertrand method [1], hydrophilic colloids (reversible and nonreversible according to Dymanskii and Simonova [2], normal soluble and nonsoluble nitrogen using a colorimeter according to Shmuk [3], activity of catalase according to Bakh and Oparin [4], and the peroxides according to Bakh and Kul'tyugin [5]. The root collars were taken along with a section of the root stem 10 cm long for analysis. The sample for analysis consisted of 50 roots. The measurements were carried out with fresh material at the beginning of the winter, in the middle of the winter, and after overwintering.

The data obtained on clover mortality (Table 1) show that fertilizers decrease the mortality of clover during the overwintering period both in the first-year and in the second-year plants. More favorable conditions for the overwintering of clover result from the joint introduction of phosphorous and potassium fertilizers. Second-year clover, both in the control variant and in the variant with the application of a single phosphorous fertilizer, died out to a great extent after the use of phosphorous and potassium fertilizers on seed.

The experimental data showed that clover plants accumulate a significant quantity of sugars in the winter organs upon entering into the winter period. The high concentration of these sugars is maintained for the winter period and it is significantly reduced only toward the end of the overwintering (Table 2).

The phosphorous and potassium fertilizers, applied as a form of food, aid in the concen - tration of sugars in the root collars and the better overwintering of this sugar concentration is also attributed to this. More favorable conditions for the accumulation of sugars result from the joint introduction of phosphorous and potassium fertilizers.

First-year clover in all periods of over – wintering retained more sugars in the roots than second-year clover. In second-year plants the concentration of sugars towards the end of the overwintering was greatly decreased. The lower concentrations of sugars towards the end of overwintering in the winter organs of first-year and second-year clover reduces the resistance of plants to unfavorable conditions.

The lower concentration of sugars in the root collars of second-year clover in the experimental variant with phosphorous fertilizer can be explained, obviously, by the fact that plants in this experimental variant were older and therefore have a smaller accumulation of sugars. Phosphorous, particularly when used on seeds, accelerates the

TABLE 3

Change in Concentration of Nitrogenous Substances in the Root Collars of Clover in Various Periods of the Overwintering (in % of dry weight)

Experimental variant	First-year clover						Second-year clover					
	Beginning of winter		Middle of winter		End of overwintering		Begining of winter		Middle of winter		End of overwintering	
	Norm.	Sol.	Norm.	Sol.	Norm.	Sol.	Norm.	Sol.	Norm.	Sol.	Norm.	Sol.
Scientific section of L'vov Pedagogical Institute												
Control (no fertilizer)	2.37	1.32	2.33	1.61	1.38	0.75	1.63	0.90	1.44	0.91	0.84	0.31
P	3.84	2.60	4.06	3.51	2.06	1.22	2.38	0.88	2.22	0.92	1.16	0.54
K	2.88	2.15	2.68	2.40	1.93	1.31	2.05	1.33	2.38	1.54	1.08	0.47
PK	4.26	3.32	4.46	3.82	2.67	1.74	2.76	1.70	2.91	1.90	1.39	0.61
Experiment at Shevchenko collective farm												
Control (no fertilizers)	2.69	1.38	2.08	1.75	1.06	0.46	1.83	0.81	1.36	0.71	0.93	0.32
P	4.17	3.02	3.94	3.31	1.82	1.10	2.54	0.91	2.29	0.87	1.09	0.39
K	3.03	2.11	2.87	2.33	1.55	0.72	2.18	1.17	1.90	1.37	1.17	0.51
PK	4.12	2.60	4.05	3.32	2.28	1.63	3.16	2.02	2.75	1.94	1.24	0.42

process of ageing. Besides this, the phosphorous fertilizers in warm winters and when deep snow falls on thawed ground increase the intensity of respiration in the clover plants; this leads to a large expenditure of sugars and to high mortality of plants from exhaustion [6].

The concentration of nitrogen and the change in the form of nitrogenous compounds during the winter period is closely connected with winter hardening of plants. Our investigations showed that the phosphorous and potassium fertilizers, both when used separately and especially when introduced jointly, increase the concentration of nitrogen in the root collars. The experimental variants into which the fertilizers had been introduced were characterized not only by a large concentration of normal nitrogen, but also by its soluble form (Table 3).

At the beginning of and during the winter a large quantity of soluble nitrogen is concentrated in winter organs. The concentration of normal and soluble nitrogen in the roots sharply decreased toward spring, but the concentration of normal and soluble nitrogen at the time of application was higher in experimental variants into which the fertilizers had been introduced than in the control variants. The presence of a larger quantity of soluble nitrogen compounds in the winter period plays an important protective role in the overwintering of plants.

Although the concentration of normal and soluble nitrogen increased under the influence of phosphorous-potassium fertilizers, its concentration was lower in the root collars of second-year clover than in first-year clover. Second-year clover receiving only phosphate nutrients applied to the seeds accumulated more normal nitrogen in the root collars than the control variants, but the concentration of soluble nitrogen in all periods of overwintering was lower. The decreased concentration of normal and soluble nitrogen in the root collars of second-year clover is one of the reasons for its poorer overwintering and high mortality.

The data of the investigation on the concentration of colloids in the winter organs of clover showed that under the influence of phosphorous and potassium fertilizers in all periods of the overwintering the normal concentration of hydrophylic colloids increases and the quantity of reversible colloids is increased 1.5-2 times. This higher concentration of reversible colloids guarantees the high resistance of cells to the unfavorable influence of low temperatures. The concentration of reversible colloids significantly decreased towards spring, but their concentration in this period was higher in variants where fertilizers had been introduced.

Reversible colloids accumulate less in the roots of second-year clover than in first-year plants, and as a result of this, the resistance of second-year plants decreased significantly.

The determination of the activity of peroxide in the root collars of first-year clover showed that its activity was higher at the beginning of winter, decreased insignificantly in January, but showed a sharp decrease toward spring. The higher activity of peroxide was observed in clover that had received fertilizer, and, in particular, in clover that had received the joint phosphorous and potassium fertilizers.

The activity of the peroxide is maintained on a moderately high level in second-year clover in the autumn-winter period; toward spring its activity decreased, but less sharply than in the first year.

The activity of catalase in the root collars of clover in the autumn-winter period did not show actual differences by fertilizer. The activity of the catalase decreased with a reduction of temperature and the change of the plants to a condition of rest; its activity increased significantly toward spring when the plants emerged from the condition of rest and resumed the growth processes. The phosphorous and potassium fertilizers aided the increase in the activity of the catalase in this period. The activity of catalase in the root collars of the clover decreased in the growth of older plants (second-year plants) in the conditions of our experiment.

Thus, phosphorous and potassium fertilizers, generally introduced jointly as a form of food, lower the mortality in clover during the overwintering period. They aid in the accumulation in the root collars of sugars, normal and soluble nitrogen, and also increase the concentration of reversible colloids. The accumulation of these substances guarantees better resistance of clover to unfavorable overwintering conditions.

On the basis of the above investigation the wide use of phosphorous-potassium fertilizer to increase the resistance of clover can be recommended to collective and state farms in the clover zone.

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HOW MANY LIGHT STAGES IN WHEAT?

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Kuperman [1] has introduced in recent years a new hypothesis that suggests that there is not one, but three light stages, one following after the other. After the stage of vernalization comes the light stage in which the primary condition is the lengthening effect of light, or the "long day".

In the third stage the primary condition continues to be the factor of light, but now the spectrum composition or quality of the light is important, and in the fourth stage light is again one of the primary factors, but its intensity is of principle importance [1]. Thus, the author's fourth stage is identical to Sapegin's third stage [2,3] and Novinkov's third stage [4], and only Lysenko divides this normally accepted light stage into two parts.

According to the information of the author, " the light stage is completed at the time of segmentation of the growth cone and the formation of spike tubercles proceeds in the following or third stage of development, separated, at the present time, by many investigators – T.D. Lysenko, V.I. Razumov, M.A. Bassarskaya, E.P. Rzhanova and others."

However, we must state that the conclusions of F.M.Kuperman cannot be considered as being sufficiently well verified.

All of the above conditions of development in the light stages, including the length of the daily light, plus one additional factor, the quality of light, of which the concentration of light with long-wave red rays is of decided importance, are necessary in order to divide the stage again.

The conclusions of F.M. Kuperman are based on numerous experiments from 1940 to 1955 using, principally, type Lyutestsens 62. The variants of the experiments were sometimes changed but the basic scheme was as follows: besides a control of normal day length, there were variants with short eight and nine-hour days excluding a) morning and evening hours, b) daytime hours, c) only the evening hours, and d) only the morning hours.

The results of the experiments serving to separate new stages were basically these: the plants could complete three stages of organogenesis on the short day from 9 A.M.-6 P.M. or from 10 A.M.-6P.M. but the plants did not form the spike protuberences and did not approach spike development as in the normal light day; however the generative development was brought about on lighting the plants for a period of four and five hours in the morning and three and four hours in the evening, although this development was slower.

F.M. Kuperman's confirmation that plants of vernalized wheat cannot carry on their development up to flowering on eight to nine hour days is erroneous, although it agrees with the conclusion of Alekperov [6] and Bassarskaya [7]. We obtained budding out not only for early-ripening types Lyutestsens 62 and Melanopus 69, but also for a very later-ripening type Ak-Bidai 630 with eight and nine-hour days in the long and carefully carried out experiments in 1944-1945. Lighting was produced for the plants from 10 A.M.-6 P.M. from 8 A.M.-6 P.M. and from 8 A.M. - 8 P.M. beginning from the time of full sprouting up to the time of budding and flowering; control plants had eighteen hours of light with lighting from electric lamps of 300 watts.

The experiments were carried out in vegetation containers in a greenhouse; exclusion of light was accomplished with light-proof black hoods for each container.

We registered differentiation of spike growth in this experiment by our scale [8,9], but for convenience in the greenhouse the comparisons were indicated approximately by the stage of organogenesis using the F.M. Kuperman scheme.

TABLE 1

Generative Development of Vernalized Wheat in Relation to the Length of the Daylight

Photoperiod, in hours	Date of occurrence of stages of organogenesis			Beginning of spike development	Vegetation period from sprouts	
	III	IV	VI		Up to VI stage	Up to spike development
Lyutestsens 62						
8	19.X	25.XI	30.I	30.IV	137	210
10	10.X	19.XI	13.I	10.III	120	176
12	3.X	25.X	25.XI	1.III	90	165
Ak-Bidai 630						
8	19.X	30.XI	30.I	17.IV	141	214
10	19.X	19.XI	10.XII	10.III	120	176
18	10.X	25.X	9.XI	29.XII	55	105

Two conclusions can be drawn on the basis of the data obtained in the greenhouse: first, there was complete budding both for the plants of the early-ripening type Lyutestsens 62 and for the late-ripening type Ak-Bidai 630 on the eight-hour day, with the exclusion of the morning and evening hours; secondly, the difference in the progress of the generative development between the variant with eight-hour days and the remainder increased to the extent of the change from the III to the VI stage of organogenesis; thus, reduction of the light day stongly retards the generative development for the entire period of the development up to the formation of anthers. On the other hand, the development of plants in most variants from the VI stage to the opening proceeded in general in an identical manner after the completion of the flowering stage.

In this way the first statement of F.M. Kuperman appears to be wrongt wheat did accomplish its generative development, although slowly, for the eight-hour day, with morning and evening hours of light excluded.

Demonstrations of the decided effect of long red waves on the generative development in recently opened stages are not conclusive. The most significant data of the experiment established in July, 1954 at the White Sea Biological Station in the 24-hour Arctic day are given in the book by F.M. Kuperman ([1] Table 23).

Light for a period of eight hours during the day from 10 A.M.-6 P.M, and during the Arctic night when the red waves are definitely dominant, has practically the same effect on the development of wheat: for Lyutestsens 62 the second stage of organogenesis was recorded in both variants, but the growth of the spikes was delayed by poor conditions of photosynthesis in the "night" hours. Retardation of the development of plants in the "night" light in the Arctic was a result of the low intensity of light and lower nighttime temperatures. (Razumov and Smirnova [10]). The shift to the morning hours (6 A.M.-2 P.M.) showed an effect on Lyutestsens 62, but light in the morning and daytime hours resulted in the same III stage of organogenesis and a practically identical size of spike for Gordenform 189.

The observed advantage in the development of wheat both in this experiment and in all F.M. Kuperman's other experiments was shown only in the variant with four or five morning and four evening hours of light. However, an explanation of the advantage of this variant does not necessarily demand the development of a hypothesis on the supplementary stages.

It is well known that it is necessary for normal flowering of any green plant to have several hours of bright light during the day. The most active wave lengths are the orange-red waves. It was established long ago that the daily curve of photosynthesis has one maximum in the morning hours and a second maximum later in the evening hours. With an insufficient normal number of light hours in the day, such as eight, for experimental plants, the optimum conditions for photosynthesis in the morning hours must be shown not only by the rate of growth but also by the development. Such a decided effect was undoubtedly shown by breaking the light period into two parts with the exclusion of the hours of the day that are least effective on the intensity of photosynthesis.

Thus, an insufficient accounting of the interconnection of the development and growth of wheat plants led F.M. Kuperman to the unfounded conclusion of the necessity for dividing a new stage in connection with the erroneous treatise of his numerous experiments. Such a position is in general unfortunate because the excellent monograph of F.M. Kuperman, "The Biological Bases of Wheat Culture", will undoubtedly be one of the basic textbooks on the biology of early grain culture and this serious methodological error could create difficulty for many young scientific workers and agronomists.

Besides this, there are no bases to revise critically the established rotation of the three primary stages of development of wheat and no necessity for any new intermediate stages.

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THE INFLUENCE OF ECOLOGICAL CONDITIONS ON THE RASPBERRY PLANT IN THE FOOT HILLS OF THE NORTH CAUCASUS

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The ecological conditions of cultivation are superimposed specific impressions on all activity of the plant. Thus, we noted in the foot hill zone of the North Caucasus that the growth of raspberry in understorey conditions increased the tempo of growth, increased winter-hardening, sharply raised the yield in relation to plants of the same type grown in open areas. The differences are so great that the agricultural value of raspberries in conditions of open areas sharply dropped. This circumstance stimulated us to study in more detail the biology of raspberry in open areas and under the forest.

The work was carried out at Maikopska Experimental Station of the All-Union Plant Husbandry Institute during 1954 and 1957. Observations were made on plants of various types of raspberry planted in 1946-1949 on open areas in the bottoms of the Shuntuk river, and on partially cleared areas in the forest on the second terrace on the north slope where raspberries were planted in 1952 as undergrowth in a partial forest. Root cuttings taken from plants in the open area served as planting material for the forest areas.

The planting conditions on these two sites differed sharply in intensity of insulation and soil moisture.

Observations made in 1954 and 1955 showed that the length of the vegetative period, growth of shoots, winter-hardening and harvest of raspberry on the open area were much less than in the forest understorey.

The length of the vegetative period and the average growth of raspberry stems were greater in the understorey conditions (Table 1). Analagous data were obtained for types Krasnaya Millera, Turner and Dezire Bryuno.

The data in Table 2 show that the mortality of stems and buds of raspberry was definitely greater after overwintering for those plants on the open areas than for those in the understorey.

According to the 1955 data, the yield of raspberry (average weight per bush, in grams) in the open area and in the understorey was as follows:

Type	Open area	Understorey
Kutbert (standard)	184	498
Gornet	53	337
Krasnaya Millera	29	92
Vislukha	139	243

We can see from the above data that the yield of raspberry is 1.7 to 6.3 times as large under the conditions of the understorey as in the open area.

The reduction of the activity of raspberry in the conditions of the open area is also shown in the data of the chemical analysis of the raspberry leaves (Table 3).

The degree of irrigation, the concentration of ascorbic acid, and the activity of the catalase in the leaves of raspberry in the open area were lower than or understorey conditions, and, therefore, the level of the activity and respiration of raspberry in the open area was lowered.

TABLE 1

Length of Vegetative Period and Size of Average Growth of Raspberry for Various Growth Conditions

Variety	Planting site	Beginning of vegetation			End of vegetation			Vegetation period, days			Average height of stems, cm	
		1954	1955	1956	1954	1955	1956	1954	1955	1956	1955	1956
Gorent	Open area	8.IV	29.III	10.IV	25.XI	12.X	27.X	231	197	201	97	105
	Understorey	30.III	17.III	4.IV	11.XII	1.XI	2.XI	256	228	213	281	181
Kutbert	Open area	10.IV	22.III	10.IV	25.XI	1.XI	24.X	221	212	198	180	148
	Understorey	30.III	7.III	2.IV	11.XII	1.XI	2.XI	256	237	215	261	223
Vislukha	Open area	6.IV	29.III	10.IV	30.XI	1.XI	22.X	238	197	196	84	77
	Understorey	8.IV	17.III	2.IV	30.XI	1.XI	24.X	236	228	206	148	177

TABLE 2

Effect of Growth Conditions on the Mortality of Stems and Buds of Raspberry in the Winter Period

Mortality of buds, %	Open areas				Understorey			
	1954–1955		1956–1957		1954–1955		1956–1957	
	Mortality of stems, number	Mortality of buds, percent	Mortality of stems, number	Mortality of buds, percent	Mortality of stems, number	Mortality of buds, percent	Mortality of stems, number	Mortality of buds, percent
Gornet	3	62.0	3	58.9	1	1.0	1	12.8
Kutbert	2	21.0	4	93.7	1	0.0	2	27.8
Vislukha	2—	21.0	3	70.1	1	2.0	1	17.4
Krasnaya Millera	2+	35.0	4+	80.1	2—	10.0	3	66.7
Karolina	1	15.0	2	35.7	1	5.0	1	4.9
Turner	1	5.0	2	58.8	1	5.0	1	3.3
Dezire Bryuno	2—	85.0	4	80.7	1	5.0	1	2.9

TABLE 3

Chemical Analysis of Raspberry Leaves (analyzed September 6, 1957)

Variety	Growing area	Dry substances, %	Monose %	Sucrose %	Ordinary sugar %	Abscorbic acid	Activity of catalase in ml O_2/6 min·gram dry wt
Dezire Bryuno	Understorey	50.06	3.11	0.45	3.56	104.0	21.2
	Open area	54.80	3.33	0.93	4.26	92.0	20.8
Vislukha	Understorey	49.93	3.22	0.67	3.89	110.0	20.8
	Open area	60.93	4.61	0.88	5.49	89.0	17.7

TABLE 4

Composition of Raspberry Stems, Percent of Dry Weight

Sort	Location	Moisture	Manose	Sucrose	Sum of the sugars	Ratio of surcose to monoso	Starch	Vitamin C in mg percent
	Date of analysis, December 6, 1955							
Gornet	Understory	41.57	4.94	1.76	6.70	0.36	1.22	11.0
	Open area	32.58	5.30	1.17	6.47	0.22	0.87	9.9
Kutbert	Understory	36.50	3.44	3.03	6.47	0.88	2.22	14.9
	Open area	32.38	3.44	2.31	5.75	0.67	1.30	11.4
	Date of analysis, February 6, 1956							
Gornet	Understory	41.08	3.06	2.81	5.87	0.92	2.36	13.8
	Open area	32.10	4.94	2.56	7.50	0.52	1.68	12.9
Kutbert	Understory	39.76	2.50	2.50	5.00	1.00	3.38	19.2
	Open area	33.0	2.84	2.10	4.94	0.74	2.05	10.8

TABLE 5

The Activity of Catalase in Raspberry Stems (isolation of O_2 in ml per 6 minutes per gram of dry weight)

Variety	Location	Data of analysis	
		December 6, 1955	February 6, 1956
Gornet	Understorey	2.7	1.5
	Open area	4.1	3.0
Kutbert	Understorey	2.8	1.6
	Open area	4.3	2.2

TABLE 6

The Change in Concentration of Water in the Stems of Raspberry in the Winter of 1956-1957 (percent of dry weight)

Variety	Location	November 9	December 15	February 16
Dezire Bryuno	Understorey	53.2	49.5	45.2
	Open area	49.0	40.1	35.0
Vislukha	Understorey	52.7	48.5	47.0
	Open area	47.9	43.1	39.3

Note: A batch of stems (from the base to top) of about 50 g were taken for double check in determining the moisture content.

The reduction of the level of activity of plants from the open area in this case is also shown by the concentration of sugars in the leaves. Generally there is a sharply decreased viability of raspberry from the open area during the overwintering period.

The reason for the winter-spring mortality of fruitful berry plants is often found in conditions of the preceding summer which weakened the plants.

Not only do the summer conditions have an effect on the winter-hardening of raspberry, but also the overwintering conditions in the open area appear to be less favorable when compared with the conditions in the understorey.

A biochemical analysis of the raspberry stems was made to achieve our goal of a more intensive study of winter-spring raspberry mortality in the open areas. The first analysis was made on December 6, 1955. The period preceding the date of analysis was essentially frost free. The short-term minimum, 14.8°, was noted only on December 1. The second chemical analysis of raspberry stems of the same types was made two months later on February 6, 1956. The air temperature dropped sharply and fluctuated from − 7.7° to − 23.3°. The results of the analysis are given in Table 4.

The data show that the raspberry stems in understorey conditions hold more water than the

raspberry grown in an open area. The plants can lose much water in the winter with strong winds and generally on sunny days, and are damaged from drying out. The significant loss of water by plant cells results in its turn in changes in the path of the physiological processes. In the first place, the dehydration changes the direction of the fermenting activity of the growing organism. The drying increases the intensity of the hydrolytic processes and retards synthesizing [1]. Our data confirm the presence of this condition on winter drying.

We can see from Table 4 that in the more dehydrated specimens from the open area the concentration of starch is lower, and this is related to its increased hydrolytic decomposition. The observed relationships of the quantity of saccharides to monosaccharides are also shown by the greater depth of the fermenting decomposition of saccharides in the stems of raspberry grown on the open area.

The results of the determination of the activity of catalase shows higher activity of this ferment in the cells of raspberry taken from the open are (Table 5).

In addition to this there is a generally sharply decreased concentration of saccharide, which L'vov [2] considers the basic respiratory substrate, in plants taken from the open area.

The lower concentration of soluble sugars and starch in the stems of raspberries from the open areas cannot negatively affect their ability to harden. The presence of winter-drying in raspberry grown on the open area is also confirmed by the data on moisture content of raspberry stems in winter (Table 6).

We can see from Table 6 that the moisture content of the stems in the open area is lower and drops more sharply during the winter than the moisture content of the stems from the plants grown in the understorey.

Raspberry plants in the open area can have unfavorable conditions as a result of winter drying, at least up to the time of frost during the winter period. In this case even a light frost can cause serious injury in the planting of the crop when it is combined with the influence of winter drying. Therefore, under the conditions of the foot hills of the North Caucasus, types less subject to winter drying can appear to be more frost resistant than other types of raspberry, although their frost resistance can be the same or lower.

A comparison of Gornet and Kutbert plants grown in the open area and in the understorey was made for illustration of the above hypothesis. In 1954-1955 the stems of plants of the Gornet variety lost 9% more moisture when planted in the open area than when planted in understorey conditions, but stems of the Kutbert variety lost only 4% under the same conditions. The mortality of stems of the Gornet variety was 3 and up to 62% of the buds when grown in the open area, but the mortality of stems of the Kutbert variety was 2 and 21% of the buds under the same condition. If the harvest of these types in the understorey is taken as 100%, then the harvest for the Gornet variety is about 16% and for the Kutbert variety, 37% when grown in open areas. We can also observe a similar picture for other sorts of raspberry. This suggests that the variety that is more subject to winter drying is less fruitful and less winter-hardened than others.

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APPLICATION OF COMPLETED WORK IN THE NATIONAL ECONOMY

MICRODOSES OF MOLYBDENUM IN GRAIN CROPS AND UNDERPLANTED GRASSES IN CONNECTION WITH THEIR PRODUCTIVITY AND PROTEIN METABOLISM

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Earlier work [1] has shown that the application of ultrasmall doses of molybdenum according to the method developed in our laboratory [2] (enriching with molybdenum of granulated superphosphate placed with the seeds when planted) can be a decisive factor in changes in the interspecies relations, can increase yields, better the nutritive qualities and increase the agricultural role of perennial legume-grass mixtures on podzol soils containing noticeable quantities of mobile aluminum.

As the investigations of Dagis and colleagues show [3], the introduction of ultrasmall doses of molybdenum (also of boron) in this manner is as effective for grass mixtures of clover and timothy as the introduction into the soil of a dose ten times as large of these microelements in normal broadcast manner. In connection with this, there is the question of the effectiveness of ultrasmall doses of molybdenum introduced in this way not only for perennial legumes and legume-grass mixture, but also for other crops, primarily for grains under which perennial grasses are normally planted. With an answer to this question as our goal in our experiments with grasses in which oats served as a cover crop, we studied the effectiveness of the indicated method both for the grasses themselves and for the oats; that is, we introduced the same fertilizer in the same doses in the rows when planting both with the oat seeds and with the grass seeds.

The details of the establishment of these experiments, the techniques of the preparation of the molybdenum superphosphate and of the methods of study of the yield, etc., are given in earlier accounts [1]. Here we will mention only that these experiments were carried out on sod-podzol soils of the experimental economic biology section of the AN, SSSR, in Snigiryakh, Moscow region, on large plots 0.15 – 0.18 hectares with two repetitions with the subsequent determination of the yields made on microplots laid out across the large plots.

In Table 1 we give the average data on the calculation for the yield of oats, and, following them, for the perennial grasses of the first year of use in three experiments put out in various years. The index of the accuracy (p) was calculated by the VIUAA method for geographical sets of experiments with fertilizers [4] for an estimate of the accuracy of the data obtained for each experiment. Some agrochemical characteristics of the soil on which the experiments were carried out are given in addition to the above in Table 1.

Analysis of the figures in Table 1 leaves no doubt that enriching the seeds planted on podzol soils poor in phosphoric acid and with clearly expressed acidity and noticeable quantities of mobile aluminum, with granulated superphosphate together with a microdose of molybdenum results in a well verified supplementary increase of grain harvest which averaged 2.1 centners per hectare in the three experiments. In addition to this we can see from the table that the relative effectiveness of molybdenum on oats is a significant indication of its effectiveness on grasses: in the first case the supplementary increases of the yield were in the various experiments 14.6, 6.5, and 7.5%, based on the control; in the second, 28.0, 59.7, and 16.6% respectively, based on the control. These differences show a significantly lower demand for the molybdenum by oats than by the grasses (especially with the legume grasses – see Fig. 1); that also explains the significant differences in the concentration of molybdenum in the legume and grassy plants [5].

TABLE 1

Relative Effectiveness of Microdoses of Molybdenum on Oats and Underplanted Grasses

Year and experimental variants	Oats			Grasses, first year			Soil characteristics (pH—colorimeter, active aluminum by the Sokolov method, active P_2O_5 by Kirsanov)
	Grain harvest in centners per hectare	Increases from fertilizers		Grain harvest in centners per hectare	Increases from fertilizers		
		in centners per hectare	in % from control		in centners per hectare	in % from control	
1954—1955							
No fertilizers with the seeds (control)	14.4	—	—	35.7	—	—	pH in KCl, 4.7; mobile Al, 3.0 mg per 100 g of soil
Granulated superphosphate with the seeds	16.4	2.0	13.9	43.1	7.4	20.7	active P_2O_5 4.2 mg per 100 g of soil
Granulated superphosphate, enriched Mo (150 g/hectare) with the seeds	18.5	4.1	28.5	53.1	17.4	48.7	
	Experimental error		$p = \pm 2.0\%$	Experimental error		$p = \pm 1.2\%$	
1956—1957							
No fertilizers with the seeds (control)	41.3	—	—	30.5	—	—	pH in KCl, 4.3; mobile Al, 4.0 mg per 100 g of soil
Granulated superphosphate with the seeds	45.3	4.0	9.7	39.0	8.5	27.9	active P_2O_5, 1.2 mg per 100 g of soil
Granulated superphosphate, enriched Mo (150) g/ hectare) with the seeds	48.0	6.7	16.2	57.2	26.7	87.6	
	Experimental error		$p = \pm 1.7\%$	Experimental error		$p = \pm 2.8\%$	
1957—1958							
No fertilizers with the seeds (control)	21.4	—	—	39.8	—	—	pH in KCl, 4.8; mobile Al, 2.1 mg per 100 g of soil
Granulated superphosphate with seeds	25.3	3.9	18.2	44.7	4.9	12.3	mobile P_2O_5, 4.1 mg per 100 g of soil.
Granulated superphosphate, enriched Mo (150 g/hectare) with the seeds	26.9	5.5	25.7	52.3	12.5	31.3	
	Experimental error		$p = \pm 1.1\%$	Experimental error		$p = \pm 1.5\%$	

Fig. 1. Change of interspecies relations in phytocoenosis of perennial grasses (clover-timothy mixture) under the effect of small doses of molybdenum superphosphate placed with the seeds on planting.
Left: 50 kg/hectare of granulated superhosphate, enriched molybdenum (150 g/hectare) placed with the seeds on planting. Excellent development of clover and poor development of other grasses are visable. Right: control. Dominant development of clover, many various grasses.

All of the experiments described above were carried on, as indicated, on podzol soils with acid reactions containing noticeable quantities of mobile aluminum. We estimate the area of these soils under cultivation to be many tens of millions of hectares; an essential increase of their productivity with the help of a microdose of molybdenum would appear to be a solution to the problem which is foremost in national economic importance. However, agrochemical science has to the present time also put forth several other methods of increasing the productivity of the indicated soils (their cultivation), foremost of which has been the systematic application of manure and lime. In connection with this, it is necessary to obtain a correct representation of the place of molybdenum in the plant nutrition system and its relation to the other, now widely accepted, methods of increasing the productive capacity of podzol soils in order to arrive at an explanation of the best manner of its use in agriculture when there is still a deficit of molybdenum. One of the links in the problem – the question of the union of molybdenum with the introduction of granulated superphosphate on planting – was brought to light in the experiments explained above and also in an earlier work [1].

This question was developed further in a special experiment in which the action of molybdenum on grain crops and on underplanted grasses was investigated on four different types of cultivated soil established prior to planting by the application of fertilizers in a plowed field intended for winter wheat with underplanted grasses. These types are as follows: 1) without prior application of fertilizers; 2) with the application of 20 T of manure per hectare; 3) with the application of 20 T manure plus 3 T of lime per hectare; and 4) with the application of 20 T of manure and 6 T of lime per hectare (corresponding to the hydrolytic acidity of the soil).

The soil on which the experiments were carried out is characterized by the following features: pH in KCl is 4.5; the mobile Al is 2.2 mg according to the Sokolov method; the mobile P_2O_5 is 1.3 mg by the Kirsanov method. Concerning the grain crop, we took the region average for the Moscow region for wheat-wheat grass hybrid 599 as being a more valuable crop than oats, more responsive to acid soil and with a higher demand for fertilizers.

Microdoses of molybdenum were applied in two ways: by enriching with granulated superphosphate designed for introduction with the seeds on planting and by wetting the seeds themselves with a solution of molybdenum salt together with granulated superhosphate following their sowing.

TABLE 2

Relative Effectiveness of Microdoses of Molybdenum on Winter Wheat (Wheat-Wheat Grass Hybrid 599) and on the Underplanted Grasses in Relation to Basic (preplanting) Fertilizer

Crop and experimental variant	Without basic fertilizer					20 T/hectare of manure					20 T/hectare of manure plus 3 T/hectare of lime					20 T/hectare of manure plus 6 T/hectare of lime				
	Harvest of grain or hay in centners/ha	General increase from fertilizer		Increase from molybdenum		Harvest of grain or hay in centners/ha	General increase from fertilizer		Increase from molybdenum		Harvest of grain or hay in centners/ha	General increase from fertilizer		Increase from molybdenum		Harvest of grain or hay in centners/ha	General increase from fertilizer		Increase from molybdenum	
		centners/ hectare	% based on control	centners/ hectare	% based on control		centners/ hectare	% based on control	centners/ hectare	% based on control		centners/ hectare	% based on control	centners/ hectare	% based on control		centners/ hectare	% based on control	centners/ hectare	% based on control
Winter wheat									—	—	1									
Control (without fertilizers with the seeds)	12.1	—	—	—	—	16.0	—	—	—	—	17.8	—	—	—	—	19.4	—	—	—	—
Granulated superphosphate with seeds	14.5	2.4	19,8	—	—	19.1	3.1	19.4	—	—	21.8	4.0	22.5	—	—	23.0	3.6	18.8	—	—
Same plus 15 g/hectare Mo by wetting seeds	16.5	4.4	36,3	2,0	16.5	20.3	4.3	26.9	1.2	7.5	22.0	4.2	23.6	0.2	1.1	23.0	3.6	18.8	0	0
Granulated superphosphate enriched with Mo(150 g/hect) with seeds	18.0	5.9	48,8	3.5	28.9	21.0	5.0	31.3	1,9	11.9	23.5	5.7	32.0	1.7	9.5	23.4	4.0	20.6	0.4	2.1
Grasses	Experimental error p=± 2.7 %					Experimental error p=± 1.6 %					Experimental error p=± 2.8 %					Experimental error p=± 1.5 %				
Control (without fertilizers with the seeds)	39.6	—	—	—	—	46.9	—	—	—	—	52,1	—	—	—	—	60.7	—	—	—	—
Granulated superphosphate with seeds	48.2	8.6	21.7	—	—	55.2	8.3	17.7	—	—	58.4	6.3	12.1	—	—	65.9	5.2	8.6	—	—
Same plus 15 g/hectare Mo by wetting seeds	57.1	17.5	44.2	8.9	22,5	60,9	14.0	29.8	5.7	12.1	63.1	11.0	21.1	4.7	9.0	70.0	9,3	15.3	4.1	6.7
Granulated superphosphate enriched with Mo(150 g/hec.) with seeds.	65.5	25.9	65.4	17.3	43.7	66,5	19,6	41.8	11.3	24.1	70,6	18.5	35.5	12.2	23.4	74.0	13.3	21.9	8.1	13.3
	Experimental error p=±1.6%					Experimental error p=±1.1%					Experimental error p=±1.3%					Experimental error p=±0.8%				

TABLE 3

The Effect of Superphosphate and Molybdenum Applied with Seeds on Planting on Protein Metabolism of Clover and Timothy in Relation to the Basic (preplanting) fertilizers (concentration of N as % of dry weight)

Basic fertilizer and experimental variant	Clover			Timothy		
	Ordinary N.	Protein N	Protein N as % of ordinary N	Ordinary N	Protein N	Protein N as % of ordinary N
Without basic fertilizer						
No fertilizer with the seeds	1.99	1.81	90.9	0.97	0.83	85.6
Granulated superphosphate with seeds	2.33	2.00	85.8	1.12	0.91	81.2
Granulated superphosphate enriched with Mo (150 g / hectare) with seeds	2.54	2.44	96.1	1.37	1.20	87.6
20 T/hectare of manure + 6 T/hectare of lime						
No fertilizer with seeds	2.24	2.08	92.8	1.01	0.90	89.1
Granulated superphosphate with seeds	2.29	2.12	92.6	1.21	1.16	95.9
Granulated superphosphate enriched with Mo (150 g / hectare) with seeds	2.72	2.34	86.0	1.67	1.37	82.1

TABLE 4

Differences in Concentration of Nitrogen and Gluten in the Grain of Winter Wheat Dmitrovskaya and Wheat-Wheat Grass Hybrids Grown under Similar Conditions of Nutrition

Type of wheat	Ordinary concentration of N, %	Concentration of protein N, %	Concentration of gluten, %		Relation of concentration of gluten to concentration of ordinary N	
			Wet	Dry	Wet	Dry
Dmitrovskaya al'borubrum	2.93	2.69	37.10	14.04	12.68	4.79
Wheat-wheat grass hybrids:						
599	2.25	2.14	25.52	9.56	11.34	4.25
1	2.41	2.27	29.60	10.70	12.28	4.44

In the first case the dose of molybdenum (in the form of molybdate of ammonia) was about 150 g/ hectare; in the second case about 16 g/ hectare. The dose of superphosphate in all cases was 50 kg/ hectare. The details of the use of these two methods of introducing ultrasmall doses of molybdenum are described in a previous note [1]. Here we mention only that when the seeds are being wetted with a solution of molybdenum salt, the molybdenum salt must not be poured on the seeds, but evenly distributed with the help of a pack sprayer while the whole wetted mass is carefully turned over. Two other variants were included in the experiment in addition to the designated variants: 1) similar doses of granulated superphosphate without molybdenum were introduced with the seeds, and 2) no fertilizers were introduced with the seeds. Early spring wheat in all variants of the experiment was fertilized with nitrogen in the quantity of 1 centner ammonium nitrate per hectare. The experimental areas were 90 square meters with six repititions. The results of the experiment are given in Table 2.

TABLE 5

The Effect of Nutrition Conditions on the Concentration of Nitrogen and Gluten in the Grain of Wheat-Wheat Grass Hybrid 599

Basic fertilizer and experimental variant	Concentration of ordinary N in the grain, %	Concentration of gluten, %		Relation of gluten concentration of ordinary N	
		Wet	Dry	Wet	Dry
Without basic fertilizer					
Without application of fertilizer with seeds	2.25	22.68	7.71	10.08	3.43
Granulated superphosphate with seeds	2.19	20.74	7.04	9.48	3.21
Granulated superphosphate, enriched with Mo with seeds	2.08	20.84	7.04	10.01	3.33
20 T/ hectare of manure					
Without application of fertilizer with seeds	2.24	22.45	7.71	10.02	3.44
Granulated superphosphate with seeds	2.16	20.60	7.10	9.54	3.29
Granulated superphosphate enriched with Mo with seeds	2.06	20.35	6.99	9.87	3.39
20 T/ ha. of manure + 3 T/hec.of lime					
Without application of fertilizer with seeds	2.24	21.3	7.67	9.51	3.43
Granulated superphosphate with seeds	2.17	20.85	7.40	9.61	3.41
Granulated superphosphate enriched with Mo with the seeds	2.07	19.01	6.80	9.18	3.28
20 T/ha. of manure + 6 T/ hec. of lime					
Without application of fertilizers with seeds	2.19	20.54	7.58	9.38	3.46
Granulated superphosphate with seeds	2.19	20.75	7.84	9.47	3.58
Granulated superphosphate enriched with Mo with seeds	2.10	19 84	7 10	9.45	3.38

TABLE 6

The Effect of Early Application of Nitrogenous Food on the Concentration of Nitrogen and the Protein Composition of the Grain of Wheat-Wheat Grass Hybrids

Type of wheat and experimental variant	Grain harvest, centners/hectare	Concentr.of ord. N in the grain, %	Concentr.of protein N,	Concentration of gluten, %		Rel. of gluten concentr.to the concentr. of ordinary N		Fractioned composition of protein N as %of ordinary N			
				Wet	Dry	Wet	Dry	Salt fraction	Alcohol fraction (gliadin)	Residue (a diff. glutein)	Ratio of gliadin to glutenin
Wheat-wheat grass hybrid 599											
20 T/ha. of manure + 3 T ha. of lime, without nitrogen	17.5	1.96	1.94	20.64	7.66	10.53	3.91	30.1	40.3	29.6	1.36
Same plus nitrogen	18.2	2.25	2.14	25.52	9.56	11.34	4.25	27.1	38.7	34.2	1.13
20 T/ha. manure + 3 T/ iha.lime + granulated superphosphate with seeds, without nitrogen	22.5	1.98	1.97	18.98	7.05	9.59	3.56	31.8	38.8	29.4	1.32
Same plus nitrogen	25.1	2.29	2.17	26.70	9.76	11.56	4.26	26.7	39.7	33.6	1.18
Wheat-wheat grass hybrid 1											
20 T/ha. of manure plus 3 T/ha. of lime, without nitrogen	22.2	2.13	1.97	23.91	8.01	11.22	3.76	29.6	38.5	31.9	1.21
Same plus nitrogen	25.7	2.41	2.27	29.62	10.70	12.28	4.44	23.2	40.7	36.1	1.13

TABLE 7

The Effect of Late Root Feeding on the Concentration of Nitrogen and the Protein Composition of Grain of Wheat-Wheat Grass Hybrid 599

Experimental variant	Concentration of ordinary N in the grain, %	Concentration of protein N in the grain, %	Concentration of gluten, %		Ratio of gluten concentration to ordinary N concentration		Fractionated composition of protein N as % of ordinary N			
			Wet	Dry	Wet	Dry	Salt fraction	Alcohol fraction (gliadin)	Residue as a difference (glutenin)	Ratio of gliadin to glutenin
Sprayed with water	2.09	2.04	24.49	8.48	11.71	4.06	31.2	42.3	26.5	1.59
Sprayed with $NH_4 NO_3$ solution	2.33	2.29	27.01	9.84	11.56	4.22	28.6	42.1	29.3	1.4

The data in Table 2 indicate conclusively that microdoses of molybdenum were effective on the original ground of fertile soil (without the application of the basic fertilizer) both for the perennial grasses and for the winter wheat. Even an insignificant dose of molybdenum of 15 g/ hectare on this ground gives a completely reliable increase in the harvest both for hay and for grain, although the increase is significantly smaller than the increase resulting from a tenfold dose of molybdenum introduced in the granulated superphosphate.

The relative reaction of the wheat-wheat grass hybrid to the molybdenum fertilizer was substantially higher than the reaction of oats in the indicated experiments. However, the significance of molybdenum for the wheat-wheat grass hybrid was very noticeably reduced from its effect on the legume-grass mixture.

The action of the molybdenum was as a rule decreased both for wheat and for grasses according to the degree of increase of cultivation of the ground. However the difference in the reaction of grain crops and grass (clover) emerged still more clearly in the above conditions than on unfertilized ground. The application of granulated superphosphate enriched with molybdenum under grasses had in all cases, without exception, an additional effect on the harvest, significantly exceeding the effect from the superphosphate itself (one and one half to two times). Even when 20 T/ hectare of manure and a full dose of lime (6 T/ hectare) is introduced into the soil, the enrichment of the superphosphate with molybdenum gave a fully reliable additional increase in the hay harvest of 8.1 centners/ hectare (13.3% more than the control) that exceeded the increase from superphophate itself by one and one half times.

The results are different in the case of the winter wheat. Although on unfertilized ground the effect on this crop of enriching the superphosphate with molybdenum exceeds (although to a lesser degree than in the case of the grasses) the effect of the superphosphate itself both on the ground with 20 T/ hectare of manure and to a greater extent on the ground with manure and 3 T/ hectare of lime, the increase from molybdenum is significantly reduced by the increase from the superphosphate itself. The effect from molybdenum is practically completely removed (increase in the experimental accuracy of the experiment) on ground with 20 T/ hectare of manure plus 6 T/ hectare of lime. Even earlier (with the combination of manure and 3 T/ hectare of lime) the effect of smaller doses of molybdenum (15 g/ hectare applied to seeds) is completely removed.

Molybdenum maintains its effectiveness in this manner both on grasses and on grain crops on podzol soils with an acid reaction and with a noticeable concentration of mobile aluminum. Its effectiveness on the grain crops is practically eliminated when these same soils are limed, while its effectiveness on grasses isstill maintained to a very noticeable degree, especially with small doses of lime. It also follows from Table 2 that if liming does not eliminate the effectiveness of molybdenum on grasses, then in turn molybdenum by no means decreases the effectiveness of liming: a larger harvest of hay (74 centners/ hectare) in the observed experiment was obtained by combining molybdenum with liming of the soil.

Finally there is one additional difference from these data in Table 2 concerning the relative importance of the application of ordinary granulated superphosphate (not enriched with molybdenum) for grasses and for the grain crop: if in the case of grasses the absolute and relative increases in the harvest resulting from the application of granulated superphosphate when the seeds are planted are as a rule decreased according to the degree of increase of culturing of the soil, then in the case of wheat they steadily maintain their significance uncorrelated with the ground conditions. This position in respect to wheat is also confirmed in other experiments carried out during the two years with wheat-wheat grass hybrids 599 and 1 on the same soils of experimental agriculture which were very poor in the availability of phosphoric acid for the plants. The combination of data that we obtained in experiments with wheat-wheat grass hybrids illustrates again the importance of the application of small doses of granulated superphosphate when the seeds are planted as a "primary highly effective link in the plant system of grain crops" [6], in particular winter wheat.

In conclusion we have the important question of the significance of molybdenum as one of the links in the system of plant nutrition for protein metabolism of grasses and wheat-wheat grass hybrids.

In an earlier work [1] it was shown that enrichment of the soil with microdoses of molybdenum under condition of molybdenum deficiency on acid soil containing noticeable quantities of mobile aluminum has a decided significance not only for the harvest of grasses, but also for the increased level of use of nitrogen entering into the plant on the synthesis of proteins. In addition to this, in regards to the legume component of the grasses (clover), a significant increase in the ordinary concentration of nitrogen in the plants was noted, so that in the combination of the effect of these two factors (the increase in the concentration of ordinary nitrogen and its more complete use in the synthesis of protein) the concentration of protein in the leaves and stems of clover increases one and one half to two times.

The indicated characteristic effect of molybdenum on the protein metabolism of grasses grown under conditions of sharply expressed molybdenum deficiency was also confirmed on grasses set out under a cover of winter wheat in the experiments mentioned above. As the data in Table 3 indicate, in this experiment, as in an earlier published experiment [1], a reduction is noted in the effect of granulated superphosphate, which was applied when the seeds were planted, on the level of use of nitrogen entering into the plant on the synthesis of protein, and there is total elimination of this depression in the synthesis of protein when the superphosphate is enriched with microdoses of molybdenum. This fact is noted only under conditions of strongly expressed molybdenum deficiency (ground without ordinary fertilizer) and is repeated both for clover and timothy.

The application of manure and a full dose of lime to the soil, while lessening the molybdenum deficiency, leads to the liquidation of the negative effect of superphosphate on protein synthesis. Under these conditions the enrichment of superphosphate with molybdenum significantly increases the concentration of ordinary nitrogen in the plants. The level of its use in the synthesis of protein is even slightly decreased, although in absolute terms the maximum concentration of protein is also noted in plants receiving molybdenum.

There is a great deal of interest in connection with the data obtained on grasses in explaining the effect of microdoses of molybdenum, together with other links in the system of plant nutrition, on the protein metabolism of wheat-wheat grass hybrid, primarily on the concentration of protein in the grain.

The extraordinary importance of this question is dictated by the circumstance that the first of our preliminary investigations, together with the many known qualities of the wheat-wheat grass hybrids (stability under field conditions and series of diseases, high response to fertilizer, etc.), showed their reduced capacity for the accumulation of nitrogen in the grain and for the use of stored nitrogen for the formation of glutenous proteins.

This can be graphically seen in Table 4 in which the concentration of nitrogen and gluten are compared for wheat-wheat grass hybrids 599 and 1 and for Dmitrovskaya al'borubrum wheat, a winter wheat suited to the Moscow region, grown on adjacent plots in similar, relatively favorable conditions of nutrition (20 T/hectare of manure plus 3 T/hectare of lime plus previous nitrogen application). We can see in Table 4 that the concentration of gluten in the grain of wheat-wheat grass hybrid 599 (also suited to the Moscow region) is practically reduced one and one half times from its concentration in Dmitrovskaya al'borubrum winter wheat, a fact that is explained both by the lower concentration of ordinary nitrogen in the grain of the hybrid and by the lower use of this nitrogen in the production of gluten.

These data also caused us to pay special attention to the question of the effect of molybdenum in connection with other links in the system of plant nutrition, to the concentration of nitrogen and gluten, and in several cases also to the protein composition of the grain of wheat-wheat grass hybrids.

In Table 5 are data on the concentration of nitrogen and gluten in the grain of wheat-wheat grass hybrid 599 in the experiment, the results of which are given in Table 2. The figures in Table 5 indicate conclusively that the nutrition conditions which were created both with the aid of preplanting (basic) and with the aid of the application of fertilizers at the time of planting, did not appear to result in an increase in the concentration of nitrogen and gluten in the grain of this hybrid under the conditions of the experiment. The figures were not compiled for molybdenum, which, applied with the seeds as in the case of granulated superphosphate, soon disclosed the tendency toward a reduction in the concentration of nitrogen and gluten in the grain in relation to the control (without the application of fertilizers with the seeds). This tendency was expressed more clearly in those cases where the molybdenum aided in the increase in grain harvest (on nonfertilized ground or on ground with 20 T/hectare of manure), and was less noticeable where the effectiveness of molybdenum on the grain harvest was not quite clear (on ground with manure plus 6 T/hectare of lime).

The indicated rule was also repeated in other experiments with both wheat-wheat grass hybrids 599 and 1. Further investigation of this question showed that for all systems of nutrition that we examined, only the nitrogenous food had decided effect on protein metabolism for the management of wheat-wheat grass hybrids.

In the experiments that we have described, the early application of nitrogenous food was introduced in ordinary ground; therefore it does not seem possible to consider its role. This was done in special supplementary experiments in which we studied, on one hand, the effect of the exclusion of early application of food on the concentration of nitrogen and the protein metabolism for wheat-wheat grass hybrids 599 and 1, and, on the other, the effect of later supplementary nitrogenous feeding in this same connection.

The results of the experiment in the first of the indicated cases are given in Table 6, from which an identical answer is seen both for hybrid 599 (for two nutrition conditions) and for hybrid 1. The exclusion of the early application of nitrogenous food in all cases reduced the concentration of ordinary nitrogen and protein nitrogen, glutens and the level of use of the nitrogen stored in the grain when gluten is formed. Using the fractionated composition of proteins, we can explain the latter case by the fact that on excluding the nitrogenous food, the portion of glutenin • in the composition of proteins of grain increases as the portion of salt soluble protein (albumins and globulins) not participating in the formation of gluten increases.

The second question, that of the effect on the protein composition of the grain of later additional feeding, was studied for wheat-wheat grass hybrid 599 grown on ground on which there was a preplanting application of 20 T/hectare of manure plus 3 T/hectare of lime on the soil, the application of molybdenum superphosphate when the seeds were planted, and early application of nitrogenous nutrients in the form of 50 kg/hectare of ammonium nitrate. The nutrients were applied at the end of the flowering period on this plot of ground to small areas of one square meter in three places separated from one another by 10 meters (three repetitions). Taking into consideration the data of Petinov and Pavlov [7] on the effectiveness of late root feeding of wheat with nitrogen, we sprayed the plants of this small area with a 1% solution of ammonium nitrate calculated at 37.5 kg/hectare or 12.5 kg/hectare of nitrogen. In every case similar small plots were set up close by and sprayed with identical quantities of water (control).

The results of the analysis of the grain as to the concentration of nitrogen, gluten, and the fractionated composition of the proteins of this experiment, are given in Table 7.

We see from Table 7 that the concentration of gluten in the grain continues to increase with the addition of late root feeding to early application, and approached 27%; that is, it is closer to the normal concentration of gluten in wheat grain of a higher quality. In addition to this, in this experiment as in earlier experiments, the additional nitrogenous nutrients led to the rebuilding of the fractionated composition of proteins to the extent of strengthening the glutenin and reducing the salt soluble fraction.

We may generalize from the data and conclude that if the application of molybdenum superphosphate with the seeds is a reliable way to have a significantly increased harvest and protein content of hay for legume grasses and legume-grass mixtures under conditons of phosphorous and molybdenum deficiencies (a very characteristic combination for many acidic podzol soils), then for winter wheat with the help of molybdenum superphosphate we can attain a very substantial shift in the harvest of grain only, but not an increase in its quality (increase in the concentration of protein, decrease in gluten, etc.)

• The fraction of the protein not extracted with the salt or alcohol solutions is taken conditionally here for the portion of the glutenin.

Nitrogenous feedings can serve as a very radical means of attaining the latter goal, and are generally used in a combination of early application and late root zone feeding.

SUMMARY

1. On acidic podzol soils containing noticeable quantities of mobile aluminum, enrichment of granulated superphosphate, designed for application with the seeds at planting, with a microdose of molybdenum and also the enrichment of the seeds themselves with small doses of molybdenum, leads to a substantial increase in the harvest both for perennial grasses and for grain crops under which these grasses are planted. However the response of grain crops to molybdenum fertilizer is significantly reduced when compared with the response of legume-grass mixtures.

2. Liming soils with a full dose of lime almost completely reduces the effectiveness of molybdenum on grain crops, while there is still a significant increase in the harvest for legume-grass mixtures when superphosphate is enriched with molybdenum.

3. Earlier observations, that the level of use of nitrogen entering into the plant for the synthesis of protein is negatively affected when granulated superphosphate is applied with the seeds at planting under conditions of strongly expressed molybdenum deficiency are confirmed. Enriching the superphosphate with molybdenum while increasing the normal concentration of nitrogen completely eliminates this depression in the synthesis of protein and even increases its intensity in comparison with the control. The concentration of protein both in the legume and in the grass component of the mixture increases almost one and one half times as a result.

Lessening the molybdenum deficiency under conditions of high doses of lime reduces the indicated negative effect of superphosphate on the level of nitrogen used for protein synthesis. Under these conditions the enrichment of superphosphate with molybdenum leads only to an increase in the normal concentration of nitrogen in the plants, which in the final analysis significantly increases the concentration of protein.

4. In the experiments with winter wheat (wheat-wheat grass hybrids 599 and 1), the use of molybdenum superphosphate, as well as the use of manure and lime, failed to increase the concentration of nitrogen in the grain and the level of its use for the synthesis of gluten proteins. Nitrogenous feeding has decided importance in this connection because under its effect both the normal concentration of nitrogen and the level of its use in the formation of gluten proteins (glutenin) in the grain are increased to the extent that the concentration of salt soluble proteins decreases. As a result, both the normal concentration of gluten in the grain and the decrease of gluten to the original nitrogen increases noticeably.

5. The use of small and ultrasmall doses of molybdenum by introducing it in granules of superphosphate and enriching seeds with it makes a very reliable reserve for further growth in productivity of agriculture on non-chernozem fields, particularly on acidic podzol soils containing noticeable quantities of mobile aluminum.

In addition to the further study of various physiological and agronomical aspects of the problem of molybdenum deficiency, it is necessary in the near future to introduce this reserve by means of organized production of molybdenum superphosphate in our fertilizer business and the supplying of molybdenum salts to agriculture for enriching seeds.

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METHODS

THE PROBLEM OF IDENTIFICATION OF ORGANIC ACID-SOLUBLE PHOSPHOROUS COMPOUNDS IN PLANTS BY MEANS OF REGULATED PAPER CHROMOTOGRAPHY

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In studying the phosphorous metabolism of plants, investigators have a special interest in the fraction of the organic acid-soluble phosphorous compounds, including phosphorous esters of carbohydrates, high-energy bound phosphorous compounds (ATP and ADP), and also a series of other highly active phosphorous metabolites*. Besides this, the data on the concentration of these substances in plants are very limited. The reason for this is connected with the inapplicability of many indirect methods developed for living objects for the identification of phosphorous esters in growing material in connection with differences in the composition of phosphorous esters and the presence in plants of substances preventing the determination of several groups of phosphorous compounds [1, 2]. This stimulated investigators to shift from indirect methods of analysis (in relation to acid and alkaline hydrolysis, fractionated precipitation of separate groups of phosphorous compounds, and others) to the direct determination of phosphorous-containing substances in the given fraction.

The basic method used for the identification of determined phosphorous esters at the present time is the method or regulated paper chromotography. We must note, however, that chromatographs of phosphorous esters as developed now are still clearly insufficient. Separate works have been carried out in the last ten years on the differentiation of artificial mixtures of several phosphorous esters which cannot be considered successful as appropriate attempts to determine phosphorous esters in extracts from biological objects. [7,8,9].

The authors of the given article established essentials for the identification of phosphorous esters in a series of plant objects (flax seeds, poppies, wheat and corn, cotton fibers) in connection with studying several questions of the carbohydrate and fatty metabolism of plants.

It is considered expedient to generalize from our experimental work in this area in view of the poor study of methodological questions connected with the chromotography of phosphorous esters and the composition of this group of phosphor compounds for plants.

Obtaining the fraction of phosphorous esters. The fractions of the phosphorous esters were obtained by standard methods [1,10]. Materials were digested with a 5% trichloroacetic acid under refrigeration**. In the resulting extract, phosphorous esters were precipitated in the form of barium salts, precipitated not less than two times, and transformed into Na salts in which form they were subjected to paper chromotography. The process of preparing standard solutions (taps) of phosphorous esters depends on their form in the original preparation. The preparations of barium salts of phosphorous esters are transformed into sodium salts for which 5 to 10 mg of barium salts are dissolved in 1 cc 0.1 NHCl, 1 to 2 drops of 10 % N_2SO_4 (avoiding the creation of an excess of salt in the solution) were added, centrifuged, rinsed with acidified water and neutralized. The final volume for the described batch was about 2 cc. The preparations of free phosphorous esters were dissolved in H_2O and neutralized with NaOH. The final volume also approached 2 cc. For chromotography, 0.01 to 0.02 cc of these solutions were used.

*In the future, for simplicity of exposition, we will handle this fraction, representing acid-soluble phosphorous compounds precipitated by barium and alcohol with pH 8.2, as a fraction of phosphorous esters.

**The extraction of phosphorous esters was carried out with an 80% boiling ethyl alcohol solution in several experiments.

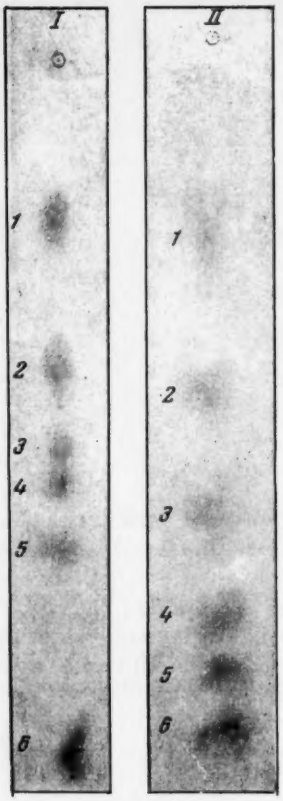

Fig. 1. Descending chromatogram of phosphorous compounds.
I) Solution, methyl alcohol (120); ammonia 25% (20); basic acid 2% (30). 1) fructoso-diphosphate; 2) glucoso-6-phosphate; 3) orthophosphate and fructoso-6-phosphate; 4) riboso-5-phosphate and phosphoroglyceric acid; 5) glucoso-1-phosphate; and 6) glycerophosphate. II) Solution, ethanol (36); butanol (30); formic acid 85% (5); and water (20). 1) fructosodyshosphate 2) glucoso-1-phosphate; 3) glucoso-6-phosphate; 4) riboso-5-phosphate; 5) glycerophsophate; 6) orthophosphate.

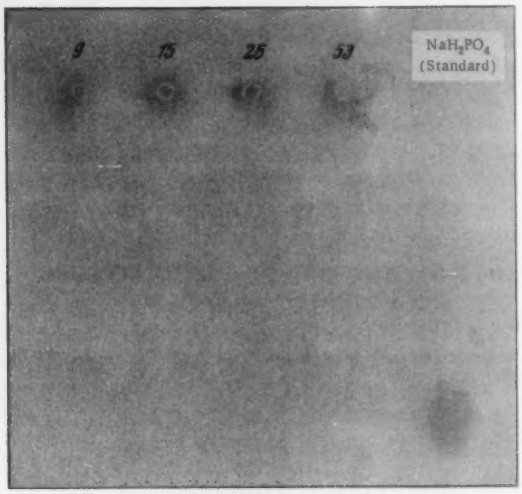

Fig. 2. Chromotograph separation of inorganic phosphorous of poppy seeds in the solution - methyl alcohol (120), ammonia 25% (20), boric acid 2% (30). The spots on the starting line match the "stationary" and below the chromatograms the "mobile" form of the inorganic phosphorous. The numbers show the age of seeds in days.

Preparation of paper and solution. Leningrad "fast" paper which had been washed with 12% formic acid and distilled water (with subsequent drying up to the complete removal of the formic acid) was used for chromatography.

Analysis of the various systems of the solutions [3-7-11-13] showed that most of these do not give satisfactory differences even for the small numbers of phosphoric acids which we studied. According to our data, better results in connection with differentiating phosphorous esters of carbohydrates, were obtained with the method: methyl alcohol, 25% ammonia, and 2% H_3BO_3 in the ratio 120:20:30 with prior washing of the paper with a 1% solution of boric acid in 60% ethyl alcohol [9] for 22 hours at a temperature of 20°C with a downward flowing solution. Separating in this system was improved thanks to the formation of complex compounds of phosphorous esters with boric acid, the size of the R_f which differentiated between them already being somewhat larger than for the natural phosphorous esters [14].

Better separation of several phosphorous esters, and also of the natural plant mixtures was also obtained in a system with the solution: ethanol, butanol, 85% formic acid, and water in the ratio 36:30:5:20.

Chromatography was carried out in the downward current of the solution for a period of twenty hours at 20°. The quantities of R_0 (ratio of the interval passed with the given substance to the path of the passing orthophosphate) are given in Table 1. (see also Fig. 1).

Fig. 3. Chromatograph separation of inorganic phosphorous in seeds of flax in the solution – methyl alcohol (120), ammonia 25% (20), boric acid 2% (30).
The numbers above the original points show the age of the seeds in days. The tap for NaH_2PO_4 is shown on the right.

Identification. The similar sizes of the R_f, and therefore the poor separation of phosphorous esters, must primarily explain the difficulties which develop when identifying these compounds. It is absolutely clear that the identification of phosphorous esters on the basis of a comparison of the rate of their movement (in terms of their R_f) is not reliable. Therefore in order to get a reliable identification of the phosphorous compounds on paper chromatograms it is necessary, in addition to the R_f, to check their relation to hydrolysis and also to use a series of other additional procedures aiding in exposing the character of the compound connected with phosphoric acid.

We used the following qualitative reactions, described in the literature, widely in our work.

1) The reaction of the deoxidation of $AgNO_3$ in an ammonia solution [15, 16] for the detection of phosphorous esters having freely reducible groups (glucoso-6-phosphate, riboso-5-phosphate, fructoso-6-phosphate, etc.).

2) The reaction with analine phthalate [15] and orthotoluidine [17] for the identification of phosphorous esters having a free aldehyde group (glucoso-6-phosphate, riboso-5-phosphate, triphosphoglycerin aldehyde).

3) The reaction with resorcin [18] and naphthoresorcin [19, 20] for the detection of phosphorous esters having a free keto-group (fructoso-6-phosphate, fructoso-1, 6-diphosphate, ribuloso-5-phosphate, ribuloso-1, 5-diphosphate).

4) The reaction with orsin [21] for the detection of phosphorous esters of pentose and heptose (ribose-5-phosphate, ribuloso-5-phosphate, sedheptuloso -7-phosphate etc.)

The reaction of the periodic oxidation [22] used normally for the determination of polyatomic primary alcohols and giving, it appears, the possibility to show not only phosphorous esters of sugars but also phosphorous derivatives of polyoxyacid and multiatom alcohols not having a reaction on carbohydrates (6-phosphoglucose acid, glycerophosphate, etc.) was checked for the identification of the phosphorous esters. However we observed that this reaction cannot be used for the identification of such phosphorous compounds because phosphoric acid itself reacts with analogous phases of multitatom alcohol, and as a result of this all phosphorous compounds render themselves identical.

Nevertheless, the reaction of periodic oxidation can be used for determination of carbohydrate components of phosphorus esters after enzyme or chemical hydrolysis of the latter.

One of the convenient methods used to determine the positions of nucleotid phosphates (AMP, ADP, ATP, HTP, HDP, etc.) on the chromatogram is a review of the undeveloped chromatogram in the ultrachemiscope [23] in which the presence of filter UFS-1 and a luminescent screen made it possible to see the dark spots of the indicated compounds on the chromatogram located in the region of 260 mμ on the spectrum.

Observation of the change of the color spot of the developed phosphorous esters in ammonia vapor can be of positive help in the identification of phosphorous esters.

A number of investigators carried out the preparation of the developed chromatograms with ammonia vapor for the elimination of the blue zone appearing soon after their development. Besides this, neutralization is necessary for the preservation of the chromatograms, because the nonneutralized chromatograms are brittle and begin to crumble as soon as 3 to 4 days after developing.

TABLE 1

Quantities of R_0 for a series of Phosphorous Esters in Two Types of Solutions

Compound	Methyl alcohol (120): 25% ammonia (20): 2% boric acid (30)	Ethanol(36):butanol(30): 85% formic acid (5): water (20)
Riboso-5-phosphate	1.08	0.90
Glucoso-1-phosphate	1.14	0.64
Glucoso-6-phosphate	0.92	0.81
Fructoso-6-phosphate	1.00	—
Fructoso-1, 6-phosphate	0.55	0.52
Glycerophosphate	1.40	0.97
Phosphoroglyceric acid	1.08	—

The preparation is carried out in a chamber saturated with ammonia vapor and continues until the background is completely bleached (usually 3 to 4 minutes). Our observations agree that the neutralizing with ammonia must be carried out not earlier than 10 to 12 hours after developing. The preparation with ammonia of chromatograms that are freshly developed or inadequately exposed after developing does not lead to the complete bleaching of the background, which instead takes on a stable yellow color. Besides this, the development of the easily hydrolized phosphorous compounds can proceed during the indicated period.

Statements on the change (or disappearance) of color spots in an atmosphere of ammonia are found in the literature. Thus, Bandurski and Axelrod[4] who were some of the first to prepare chromatograms with ammonia for removal of background color, noted that the indicated preparation led to the removal of background while at the same time all spots of phosphorous esters were retained. Such a statement is incorrect, as will be seen below. On the other hand, there is an indication [24] that preparation of ammonia leads to the disappearance of spots of hard-to-hydrolize phosphorous compounds while at the same time the spots of easily hydrolized phosphorous esters are retained. Our data on this question concur in essence with the data of the above authors. By their behavior in a NH_3 atmosphere the color reducing phosphate-molybdenum complexes are divided into two groups, one of which represents the phosphorous esters, the molybdenum complexes of which do not disappear in an ammonia atmosphere but only change their color, and the other represents compounds, the molybdenum complexes of which disappear in an ammonia atmosphere. The inorganic* phosphorus, and also glucoso-1-phosphate, ATP and ADP, that is, all phosphorous compounds easily separable in an acid medium to orthophosphoric acid are related to the first group.

The more hard-to-hydrolize phosphorous compounds, glucoso-6-phosphate, fructoso-6-phosphate, phosphoglyceric acid, fructosodiphosphate, glycerophosphate and inosinphosphoric acid, are put in the second group. Table 2 shows the color of the phosphomolybdenum complexes of various phosphorous compounds on the chromatogram as soon after developing as with the preparations with ammonia vapors.

The noted differences in the behavior of reduced phosphomolybdenum complexes of various phosphorous compounds are evidently a result of the different nature of the color products. In the case of inorganic phosphorous, and also easily hydrolized phosphorous compounds (group 1) it appears that the color is a result of, first of all, the separated (on exposure to ultraviolet light) inorganic phosphorus.

The fact that the organic, easily hydrolized phosphorous compounds are somewhat differing in color with molybdenum acid complexes from the orthophosphoric acid is thought to be the effect of the organic component of the phosphorous compound. Thus the color of spots on the chromatogram for organic compounds of this group is accumulated from the yellow-green color, which is the result of the inorganic phosphorus, and from blue, which is caused by the organic component of the molecule. The latter disappears in an ammonia vapor as a result of which spots of all compounds of the first group have a similar color dependent primarily on the inorganic phosphorus after holding in a NH_3 atmosphere.

* Phosphocreatine reacting with the molybidic acid reagent similarly to orthophosphate is related here.

TABLE 2

Color of Spots of Phosphorous Esters on Paper Chromatograms and Its Change in Ammonia Vapor

Compound	Color after developing	Color after holding chromatograms in an atmosphere saturated with NH_3
Orthophosphate	Yellow-green	Grey-blue
Glucoso-1-phosphate	Greenish blue, with the reverse side of the chromatogram yellow	Grey-green
ATP	Blue with reverse side of the chromatogram yellow	Grey-blue
ADP	Blue, with reverse side of the chromatogram yellow	Grey-blue
Glucoso-6-phosphate	Blue	Disappears
Fructoso-6-phosphate	Blue	Disappears
Riboso-5-phosphate	Blue	Disappears
Phosphoglyceric acid	Blue	Disappears
Phytin	Blue	Disappears
Fructosodiphosphate	Blue	Weak, changing to grey
AMP	Blue	Disappears

The compounds of the second group give colored hard to-hydrolize products with molybdenum, in essence, in relation to their organic components which also explains the bleaching of spots in a NH_3 atmosphere.

It must be noted, however, that the complete disappearance of spots of hard-to-hydrolize phosphorous esters does not occur in the ammonia vapor. A weak greyish film, weakened, it appears, by traces of inorganic phosphorus separated at the time of exposure under a quartz mercury vapor lamp, remains on the place of the spot.

Specificity of Method. The question of the specificity of Hanes reaction on orthophosphate must be dealt with separately. The basis of this light reaction is the reaction of the reduction of molybdic acid with the formation of what is called "molybdenum blue". There is an indication in the literature [25] that the formation of molybdenum blue can also take place in the absence of phosphorous under the effect of the various reducing agents themselves in optimum pH conditions (0.2 N of sulfuric acid). The phosphoric acid in this plays only the role of a catalyst, speeding the reaction of reduction many times. The indicated considerations lead to the idea that not only phosphorous compounds, but also other substances that have reducing properties and are present in plant extracts can give colored spots on paper under the developing conditions listed above. In part, we established that carbohydrates can also give molybdenum blue when developed in the manner indicated above. However the spots originating like this are easily distinguished from spots of phosphorous ester by the character of the color and the time that they develop.

Carbohydrates appear as brown spots with a violet hue only after 6 to 8 hours after the preparation of the chromatogram with Hanes reagent and exposure to ultraviolet light. They are fairly well distinguishable from spots of phosphorous esters. Their maximum color is approached after 36 to 48 hours at the time that the phosphorous compounds react with molybdic acid, generally at the time the chromatogram is exposed to ultraviolet light. These spots practically disappear completely (leaving only a yellowish trace) under the influence of NH_3 vapor. These groups of compounds normally do not coincide by their R_f on the chromatograms because of the more rapid movement of the carbohydrates. The precipitating of barium salts, which is four times as fast, practically completely removes the carbohydrates from the fraction of the phosphorous esters.

We established that hydroxy acids also give blue spots for the above described means of developing which are outwardly little different from the spots of hard-to-hydrolize phosphorous esters. Polyhydroxy acids, for example gluconic acid, give a more intense color for this than monohydroxy acids (lactic acid). The di-and tricarbonic hydroxy acids have more complex rules. Thus, citric acid containing three carbon hydroxy acid to only one oxy group gives, when developed, an intense color that could be connected with the oxidizing part of the

carboxy acid group in the hydroxy group in the conditions of developing. In connection with this, it is clear that the contamination of the chromatography extract with the hydroxy acids can greatly increase the difficulty of identifying phosphorous esters on paper.

Some inorganic substances can also give azure spots after being developed by the Hanes and Isherwood method. Thus, there is an indication [26] that ammonium sulfate possesses such a characteristic. The latter forms on paper in place of the localization of sulfates after developing the chromatogram. The fact that the color of these spots is retained in the ammonia atmosphere makes it possible to distinguish these azure blues from the green hue of the spots of phosphorous esters. Another characteristic feature of the spots stipulated by ammonium sulfate is the progressive strengthening of the color after developing. As a rule, these spots are very weak immediately after developing, and are sometimes completely invisible. Then their color gradually increases and approaches its maximum 20 to 24 hours after developing. Inasmuch as small quantities of Na_2SO_4 are practically always present in the fraction of sodium salts of phosphorous esters, azure blue spots stipulated by the sulfate are always present on the chromatograms. These spots are located on the level of the glycerophosphate and below the inorganic phosphorus and the majority of phosphorous esters on the chromatograms in which the distillation proceeds by means of a descending current of an alkaline solution.

Sulfates move more slowly than inorganic phosphorus in acid solutions and their position on dispersion on the chromatogram usually coincides with the position of the organic phosphorous compounds. In part, in the acid solution, ethanol-butanol-formic acid-water (see above) the spot stipulated by sulfate coincides with the position of glucoso-1-phosphate. According to our preliminary data, not only sulfate, but also several other inorganic compounds give spots with similar characteristics on the chromatograms after developing.

Investigation of plant material. The application of the indicated methods for the analysis of seeds of a series of oil and starch plants makes it possible to establish several characteristic features in the quality of phosphorous esters of seeds. It seems to us that several of the rules found have a more ordinary significance and their significance can be beneficial in work with other biological objects. In connection with this, it would be expedient to leave the other work in order to give a presentation of the phosphorous esters found in plants, and also of the difficulties and surprises arising from the change from chromatography of artificial solutions of taps to the determination of phosphorous esters in plant extracts. First of all the significant similarity in the quality of phosphorous esters of different plants and the equality of this group of phosphorous compounds ADP, glucoso-6-phosphate or glucoso-1-phosphate, phytin, and inorganic phosphorus) must be noted. The spot of inorganic phosphorus was prevalent as is true in immature seeds. Other phosphorous esters were present in a significantly smaller quantity. Glucoso-6-phosphate was the most universally occurring phosphorous ester in the study seeds, although its concentration was very low.

Thus, even in immature seeds where the quantity of glucoso-6-phosphate was smallest, the spot of this ester on the developed Hanes solution chromatogram was significantly less than the spot of inorganic phosphorus. The developing of glucoso-6-phosphate by the carbohydrate component (see above) succeeded only a significant (4 to 5 times) increase in the quantity of the solution placed on the chromatogram. It is necessary however to note that even in this case the spot of glucoso-6-phosphate developed by means of an orthotoluidine solution was weak. The indicated case is connected with the less sensitive reaction of the developing of the carbohydrate component in relation to the Hanes reaction on phosphorus. Thus the indicated difficulty explains the incorrect conclusions on the presence of hexophosphates in ripening seeds of flax that was made earlier [27].

From a number of different hexophosphates, we observed only glucoso-1-phosphate in the grain of corn and wheat taken during the phase of milk ripening. The presence of glucoso-1-phosphate in the indicated objects is connected, it appears, with the intense synthesis of starch taking place in them. Hexophosphates disappear in later stages of seed ripening and the seeds begin to accumulate phytin. The relative intensity fo the spot for inorganic phosphorous is also weakened. We did not observe the extent of the formation in the seeds of fructoso-6-phosphate, fructoso-1,6-diphosphate, phosphoglyceric acid, glycerophosphate, riboso-5-phosphate and other phosphorous esters of pentoses. It appears that in the maturing seeds these compounds are subjected to intensive metabolistic transformation, in connection with which their accumulation in a noticeable quantity is not carried out. It appears that a characteristic feature of the investigation of plants is the extremely narrow concentration of nucleotid phosphate in them. We observed from a number of this broad group of compounds only adenylic derivatives in the form of ADP. However, the possibility that we would have also observed other nucleotid phosphates on a higher concentration of the pyrophosphate fraction is not excluded. The concentration of the

indicated compounds was so small that their presence on the chromatogram was observed only with the aid of an ultrachemiscope with ultraviolet light in range 260 mμ (see the work of Venkstern and Baev [28] in connection with the details of chromatographic difference of nucleotids).

What quantity of plant material must be taken for conducting chromatographic analysis of the fraction of the phosphoric esters? The minimum original portion must be 2 g (dry weight) for seeds. For the final dilution of the 2 cc, from 0.02 to 0.06 cc are placed on the chromatogram in relation to the object and phase of development of the seeds. However the original portion must be significantly increased for complete determination of the quality of the fraction of nucleotids and also of phosphorous esters present in the seeds in trace quantities.

Observations which are very interesting, although still not fully explained at the present time, were made in connection with the status of inorganic phosphorus in the study objects. It was established that the greatest part of the inorganic phosphorus in several objects and practically all orthophosphate in separate cases remain on the starting line of the chromatogram after distillation in alkaline solutions and do not coincide with the position on the chromatogram of the tap of orthophosphate. We observed this picture on chromatography of phosphorous compounds from the seeds of flax and also for poppy seeds taken several days after the flowering of plants (see Fig. 2 and 3). Inorganic phosphorus behaved normally in other cases, for example in sunflower seeds, and its position on the chromatogram coincided with the position of the tap. The fact that seeds of different plants are distinguished by the status of their inorganic phosphorus suggests that the presence of similar phosphorous compounds that are slightly mobile in alkaline solutions apparently are not artifacts originating on chromatography.

It is interesting to note that not only various plants, but various organs of the same plant can be separated by the status of inorganic phosphorus accumulated in them. The seeds of poppy can serve as a typical example (see Fig.3). All inorganic phosphorus is in a nonmobile form in poppy seeds taken from 6-day-old pods. A spot of mobile orthophosphate appears in tests with seed taken from 9-day-old pods, although the greater part of the orthophosphate continues to be located in a nonmobile form. The intensity of both spots are equal and then begin to decrease gradually according to the degree of further ripening so that the maturing seeds contain only traces of inorganic phosphorus.

All of the above leads to the fact that the relation between mobile and nonmobile forms of phosphorus depends on some internal reasons, primarily, it appears, on the physiological state of the organism (or organ).

The question concerning the form in which the nonmobile phosphorous exists remains open for the time being. It can only be asserted that it is not simply a slightly soluble inorganic phosphate (type tricalcium phosphate). This is indicated by the preservation of a small degree of mobility of the given compound after passing the study solutions through a column with cations.

Other characteristic movements are observed in acid system solutions. It appears that inorganic phosphorus moves in them with a single spot and has a quantity of R_f equal to the tap of orthophosphate.

Knowledge of the modern literature on the chromatographic study of phosphorous compounds from different objects themselves shows a deficiency of work in which the indicated anamoly in the behavior of inorganic phosphorus was noted. However, similar regularities were observed in the work of Sisakyan and Veinova [29]. The authors established, when studying the metabolism of mulberry silkworms at different stages of metamorphosis, the presence of a high molecular complex similar to inorganic orthophosphate showing on the chromatogram. The compounds found in all alkaline solutions were not displaced from the starting line. The indicated complex was displaced from the starting line in acid solutions; however, the authors gave no report on the coincidence in these conditions of the phosphorus entering into the complex with the spot of the orthoposphate. It is difficult to speak at the present time about the identity of compounds which, on the one hand, we observed, and on the other, Sisakyan and Veinova observed. We can only state that the "nonmobile" phosphorus that the above authors observed actually enters into the composition of the organic complex, then an analagous conclusion cannot be made relative to the forms of phosphorus that we found, because all attempts to observe the organic compound connected with orthophosphate were not successful.

The presence of a similar compound slightly mobile in an alkaline solution (n-propanol, 25% NH_3, and water in the ratio 60:30:10) was also observed by T.V. Venkstern (in a personal note) in a trichloroacetate extract of nuclear erythrocytes of pigeons. As we also observed, this compound coincided with orthophosphate on transchromatography in an acid solution (40% trichloroacetate, isopropanol, and 25% NH_3 in the ratio 40:280:1.2:80).

The indicated examples show that the "nonmobile" fraction of phosphorus, which appears to be similar to orthophosphate, is encountered not only in the seeds of higher plants, but also in a series of other biological objects and it is possibly quite widely spread throughout natural life. The question of the status of phosphorous in the cells is more complex than it has been supposed until recently.

SUMMARY

It is clear from what has been said that the method of regulated paper chromatography of phosphorous esters is still suffering, at the present time, from a number of essential deficiencies, of which the important ones are the difficulties in separating on paper and also the identification of organic phosphorous compounds. Identification only on the basis of the size of R_f is unreliable. It is necessary to establish the nature of the organic component for correct identification in most places. Several quantitative reactions which can be used for this are presented in this article.

The observation of the change in color of spots showing phosphorous esters in an ammonia atmosphere is recommended as an additional means for the identification of phosphorous esters.

The inadequate specificity of the method of developing by the Hanes reaction cannot serve as a basis for rejecting it. However, careful cleaning of the plant extract and knowledge of the character of the spots which indicate other nonphosphorous compounds is necessary when using this method.

In spite of the indicated imperfections in the method of paper chromatography of phosphorous esters, its use in the analysis of acid-soluble phsphorous esters of plants makes it possible to explain the composition of phosphorous esters of seeds and its change on ripening of the latter. Use of the chromatography method for analyzing phosphorous compounds in plants forces another look at earlier, apparently clear, questions on the status of phosphorus in the cell.

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• Russian translation.

•• See English translation.

BOOK REVIEW

P.A. Genkel'. Plant Physiology and the Elements of Microbiology. Government Educational-Pedagogical Publishing House, Ministry of Education, RSFSR, Moscow, 1954, 464pp.

Serious attention is given by textbooks and special literature to the independent work of students at a time when the work of the upper school is being reorganized to better the quality of the graduating specialists. Textbooks for the upper school acquire a basic importance in connection with this. At the present time it is necessary to increase significantly the quantity of books published for the upper school, and it is also necessary to increase the requirements concerned with the quality of textbooks and their format.

The publication as a textbook of "Plant Physiology and the Elements of Microbiology", written by the well-known Soviet researcher in the area of plant physiology, Pavel Aleksandrovich Genkel', is very opportune.

The reviewed text consists of the following sections: introduction, twelve chapters, a list of special and basic literature, subject index and author index. The introduction contains short accounts of the subjects and problems of plant physiology, the relation of the organism to surrounding conditions, the difference between living organisms and nonliving objects, the relation of phylogeny to ontogeny, research methods and applications in plant physiology. The first chapter contains accounts of the basic questions of plant development; the second surveys the questions of cell physiology; the third contains morphology and physiology of bacterial organisms;the fourth discusses the water metabolism of plants; the fifth discusses assimilation of carbon by the plant; the sixth, the transformation of substances in the plant organism; the seventh, the assimilation of mineral substances and nitrogen by the plants; the eighth, fermentation and respiration, the ninth, growth of plants; the tenth, plant movement; the eleventh, periodic phenomena in the life of plants; and the twelfth, the physiology of fertilization and heredity. There is no special chapter devoted to the question of the resistance of plants;however, the author is able to give an account of these questions in those chapters of the text in which there is some connection with the material.

The arrangement of material in the chapters and its sequence are both done correctly and well. It is proper to make only the following remarks here. It seems to us that in the case of a course in plant physiology, it would be better to start with the section on the physiology of growing cells and not with the section on the development of plants. The latter section can only be properly and significantly treated later, and in fact, prior to the chapter on the growth of plants. The question of the growth of the cell wall is discussed not in the chapter on the transformation of substances, but in the chapter on the growth of plants, the chapter in which the question of the three phases in the growth of cells is examined. Incidentally, it must be noted that the author treats this question only extremely sparingly. There should be a more detailed examination of the three phases in the growth of cells and, in passing, a glance at the growth of the cell wall, because these questions are very important for a proper understanding of the process of growth. Descriptions of the method of paper chromotography both with the early and with the later material are not tied together; it must either be connected or it must be transferred to the chapter on the transformation of substances in the plant organism. The author does not sufficiently cover the much more important question of the rate of diffusion through the stomata (pages 104 and 185). The scheme of diffusion of water vapor from the open vessels and through the finely pierced lamella must be explained. This could be done without increasing the volume of the text, for example, by excluding Fig. 70 which would not affect the clarity of the account. It is not possible to understand well and master the material on the stomatic regulation of transpiration and the inflow of carbon dioxide through the stomata in the leaves without a clear understanding of the phenomenon of diffusion. There is a brief mention on page 367 of the Krebs cycle, but the scheme is not given. The student is not in a position to analyze this cycle independently without this scheme.

The author strove to show in the textbook the continuous and diverse connections of the basic divisions of plant physiology and microbiology with practical agriculture. The author failed to show conclusively that plant physiology is one of the scientific bases of national agriculture.

The text is written on a high scientific level and it reflects in a proper manner the contemporary state and the perspective for the further development of plant physiology and microbiology. A great deal of factual material is put forth by the author in a concise, but clear and simple, form. However there are some unfortunate terms in the text. For example the author writes on page 42 that "the coagulant must be distinguished from the gelatinous substance". From this phrase the student might reach the incorrect conclusion that the coagulant is in contrast to gelatin. Along this line, chilling is the reason for coagulation of many hydrophylic colloids.

We find, unfortunately, errors in the book which are not included in the list of errata. Thus, for example, the neutrons in the reaction are written $_1H^1$ instead of $_0H^1$ on page 242; there is an error on page 168 in the formula for pyrrole and one valence is omitted in the formula for chlorophyll; there is an error in the formula for arginine on page 204. Not all tables and figures are cited in the text. Not all tables have titles.

The presence in the textbook of a large number of graphs and tables will help the student acquire habits for understanding correctly and analyzing properly numbered and graphic material.

One of the decided merits of the textbook, especially in connection with strengthening the independent work of students, is the fact that every chapter is clearly divided into a series of questions that have headings.

It would be desirable when the book is reprinted to have a list of the cited works at the end of each chapter. This would allow students interested in these or other questions to become better acquainted in detail with the original works.

The book by P.A. Genkel' is of undoubted value as a good textbook for the pedagogical institutes, in spite of several deficiencies.

N.N. Ovchinnikov

CURRENT EVENTS

IMPRESSIONS OF THE WORK OF THE PHYSIOLOGY SECTION OF THE BOTANICAL INSTITUTE IN HALLE (GDR)

Investigations in the field of plant physiology in progress at the Botanical Institute in Halle under the direction of Professor Motes, have a great deal of interest for plant physiologists and biochemists, both from the point of view of the subject of the problems and in connection with the features of the approaches to their solution. In this article, I am giving my impressions of the physiology section of the Botanical Institute formed as a result of two months of work at the Institute during a leave in the GDR.

The work of the entire section is directed toward the study of two main problems, the normal mechanisms of nitrogen metabolism in plants and the biosynthesis of alkaloids. We will examine in more detail the investigations in the area of nitrogen metabolism of plants. The investigations are carried out on a very broad front: the nature of the primary accumulators of ammonia, the mechanisms of the rotation of nitrogen in the plants and, finally, questions connected with the synthesis of protein. The distinctive characteristic of these investigations is that the analyses were carried out on a very large number of different items in the systematic relationships of plants. Thus, for example, Reuter examined the composition of the stock of nitrogenous compounds separated in 166 plants representing 48 families. It was ascertained from this that glutamine and asparagine, and also the amino acids appropriate to them, are definitely not the basic form of the stock of nitrogen for all plants. For many plants this role is played by such compounds as citrulline, arganine, proline, and acetylornithine. Therefore, the composition separating the stock of nitrogenous compounds was very often a systematic feature. For example citrulline was characteristic for the entire family Betulaceae; acetylornithine for Fumarioideae; proline for Papilionatae; and, as Engel'brecht, showed earlier, ureides, allantoin and allantoic acid play similar roles for many plants. The above example shows that a union of biochemistry and systematics is very fruitful. It allows an appraisal of the role of these or other compounds in the plant world, showing the specific item in the metabolism of substances systematically for different plants, and, in addition to this, puts excellent tools into the hands of the experimenters for explaining biosynthesis of separate nitrogenous compounds.

Another distinctive investigation which is being carried out under the direction of Professor Motes is a deep physiological approach to the nitrogen metabolism of plants. In this connection Professor Motes follows the tradition of the outstanding Russian scientist, D.N. Pryanishnikov. Engel'brecht showed, while studying the nitrogen metabolism of plants by the degree of their development and also in relation to different conditions of mineral nutrition and by the extent to which they provide the plants with carbohydrates, that allantoin plays a role in a number of plants, maple for example, analagous to the role that was demonstrated by Pryanishnikov for asparagine and glutamine. Thus, Engel'brecht established that allantoin serves in a harmless form, entering from without and separating on decomposition of the protein ammonia. In addition to this, allantoin fulfills a role as a reserve and a transportable form of nitrogen, and also serves as its source for new biosynthesis. Reuter obtained analagous data in connection with citrulline.

Not only is the physiological role of nitrogen accumulators in plants studied at the Botanical Institute in Halle, but also their biosynthesis; for example, Reuter is studying the biosynthesis of citrulline. Besides this, questions connected with the biosynthesis of protein are being investigated. Doctor Byotger is studying in part the primary activation of amino acids, preceding the synthesis of a peptide bond, and Vol'gin is examining the interconnection of the synthesis of protein with the metabolism of nucleic acids.

The investigation of Professor Motes in the area of the cycle of nitrogenous substances in plants is widely known. Much attention is given in these investigations to the specific role of the roots in the nitrogen metabolism of plants. Those methodological approaches such as the sterile culture of isolated roots, culture of isolated

rooted leaves, grafting, collection and analysis of sap, and treatment of leaves with different physiologically active substances are used in studying this question. It was shown as a result of these investigations that the roots play a specific role in the nitrogen metabolism of plants and that they cannot be replaced by feeding plants with mineral salts, carbohydrates, amino acids, and their amides, protein hydrolyzates, sap taken from the roots that were removed, or with coconut milk, which aids in the better development of isolated cells. At the present time these questions continue to be investigated using marked nitrogen (N^{15}).

Two items must be mentioned relative to the organization of the investigative work: first, the fruitfulness of using specialists with biological and chemical training in the work on one topic, and secondly, the large number of well-trained personnel in the institute who greatly increased the work productivity of the scientific workers and thus shortened the time necessary for solving experimental problems.

I would like to emphasize in conclusion the hospitality of the German scientists, the friendly atmosphere and the constant help in my work that I received during the entire leave.

O.N. Kulaeva

NOTICE

The Biological Institute of Czechoslovak Academy of Sciences notes that the journal "Biologia Plantarum" will be published starting in 1959. The journal will publish works in various languages in the area of experimental botany.

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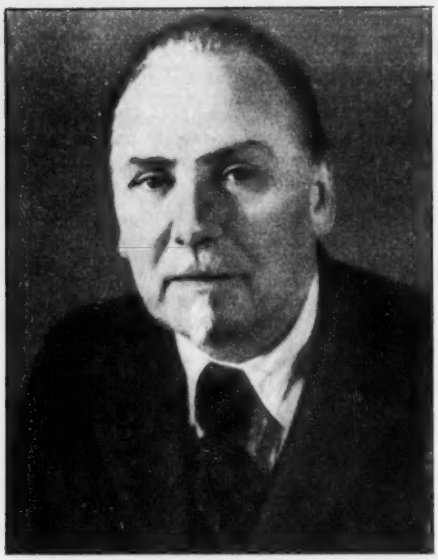

SERGEI DMITRIEVICH L'VOV

Sergei Dmitrievich L'vov, a corresponding member of the Academy of Science of the USSR and a professor at the Leningrad State University, died in Leningrad on January 6, 1959, after a long illness.

The deceased was one of the few remaining representatives of the old generation of plant physiologists, a group composed of independent scientists who worked even before the revolution.

Sergei Dmitrievich was born in 1879 in Kazan into the family of a secondary school teacher; he finished the Saratovskii Gymnasium with a gold medal in 1897 and entered Moscow University in the same year, but transferred to Petersburg University in 1898.

The life of Sergei Dmitrievich as a scientist was not completely ordinary. He was expelled soon after entering the university and sent from Petersburg for participating in a student agitation that encompassed a whole series of upper educational institutions throughout the country.

Sergei Dmitrievich entered the Social-Democratic organization after returning to Petersburg and he took active revolutionary propaganda to the pottery workers in the factories on the Nevskii gates.

He was arrested in the spring of 1900 and put in prison where he spent more than a year, whereupon he was sent in an administrative order to the former Vyatskii District for three years. Sergei Dmitrievich was able to enter again into the first course of the biological section of the physicomathematical faculty when he returned from exile, and he finished the university in 1911 in plant physiology, having fulfilled special work under the direction of Academician V.I. Palladin, and was then established in the faculty for preparation for the professorial rank.

Sergei Dmitrievich served in succession in the teaching positions of assistant from 1915-1924, as lecturer from 1924-1931, and he was named head of the chair of plant physiology as a professor, after the death of Academician S.P. Kostychev, from 1931 up to the autumn of 1957.

In addition to his work at the university, Sergei Dmitrievich concurrently undertook scientific and educational work in several other institutions: he worked in the Main Botanical Garden, now the BINE of the Academy of Sciences of the USSR, as senior educational specialist in the plant physiology section from 1920 to 1936; he lectured in the Higher Women's Natural Sciences courses and there directed the chair of botany after the departure of Academician V.L. Komarov. In 1934 the Presidium of the Academy of Sciences of the USSR gave Sergei Dmitrievich the degree of doctor of biological sciences without requiring a dissertation.

Sergei Dmitrievich was elected a corresponding member of the Academy of Sciences of the USSR in 1946 by the Biological Section.

Sergei Dmitrievich began his scientific activity in 1911 as a student of Academician V.I. Palladin; under him he developed, and maintained for his whole life, an interest in the question of the physiology of plants, particularly in the metabolism of substances. Sergei Dmitrievich's range of interests were characterized by this great breadth and timeliness that found its expression not only in a whole series of experimental investigations, but also in scientific surveys devoted to the leading problems of this science.

All of the apparent external variety of scientific interests have their purpose; Segei Dmitrievich devoted his attention to the development of the physiological reason for these or other biochemical reactions or processes performed in the plant organisms. His work could be classified according to the following problems: earlier works carried out with V.I. Palladin or under the effect of his ideas (1911-1921), devoted to the problem of fermentation, respiration of plants and to the work of enzymes in those processes. Sergei Dmitrievich also devoted many years of work to the study of the physiology of the formation of oils in plants (1933-1946) and to the physiological and biochemical features of leaves in connection with their layered arrangement. Work related to the question of the biochemical bases of drought-resistance must also be included here.

He completed several works on the chemistry of the formation of organic acids for molds in the first half of the 1930's. Sergei Dmitrievich developed a great interest in the question of the physiological significance of vitamin C for plants.

His work after the war was devoted to the study of the physiological role of sugar, especially in the process of ripening and over-ripening of fruits.

The basic conclusions of prewar experimental works and his opinions on several important physiological processes of plants and on the features of their metabolism of substances were presented in S.D. L'vov's VIII Timiryazev reading in 1947 which was devoted basically to the historical developments in the study of plant respiration [1].

The breadth of Sergei Dmitrievich's scientific interests and his erudition found their expression in a series of his survey articles on modern questions in the physiology and biochemistry of plants. Some of these are given here:

The theory of respiration of V.I. Palladin in light of new improvements in biochemistry [2], 1924.
Contemporary theories of photosynthesis [3], 1925.
The spread of irritations in plants [4], 1925.
New data on the chemistry of the formation of organic acids in plants [5], 1937.

In their time, in the 1920's and 1930's, these surveys had a very substantial and timely significance for the knowledge of a wide circle of young scientific workers with his attainments and ideas of the physiology and biochemistry of plants, not only for native plants, but also in general for foreign plants.

Sergei Dmitrievich carried out the large work for the preparation of a third printing of S.P. Kostychev's course "Plant Physiology", done as a series of supplements on the important divisions of physiology.

The characteristic features of S.D. L'vov's survey articles, apart from their valuable contents, were their beautiful, lively literary language which was rich, well-constructed and had generalized factual material put forth in a scientific manner.

The performance of Sergei Dmitrievich as a professor lecturer was above average. The broad biological erudition, the skill clearly put forth in the intricate questions of physiological science, his thorough analysis of the facts, and their interesting generalizations in combination with his literary address long ago gave Sergei Dmitrievich a merited reputation as an excellent lecturer and speaker.

His scientific and pedagogical activity did not divert Sergei Dmitrievich from the questions of public political life. He always showed a sharp, lively interest in this, always from the position of a convinced and consistent patriot. This was apparent especially during World War II. In spite of persistent suggestions and the possibility to evacuate blockaded Leningrad, S.D. L'vov left only at the end of the summer of 1942, having already undergone the difficulties of the blockade with the populace which remained.

Sergei Dmitrievich had a lively interest in the events of internal and foreign political life literally up to the last hours of his life. With the passing of the deceased, the fields of domestic physiology and plant biochemistry lost one of their old and prominent leaders.

S.V. Soldatenkov

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[2] S.D. L'vov, Advances in Biological Chemistry, 1924.*

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* In Russian.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for Experimental Morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechénova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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